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NONCARCINOGENIC EFFECTS OF CHROMIUM:

UPDATE TO HEALTH ASSESSMENT DOCUMENT

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ABBREVIATIONS

AAS	Atomic absorption spectroscopy
AIRS	Aerometric Information Retrieval System
Cr(III)	Trivalent chromium
Cr(VI)	Hexavalent chromium
DPC	Diphenyl carbazide method for Cr(IV) analysis
EPA	U.S. Environmental Protection Agency
ETA-AAS	Electrothermal atomic absorption spectroscopy, synonymous with graphite furnace AAS
FEFx	Forced expiratory flow, x indicates amount already exhaled
FEVt	Forced expiratory volume, t indicates duration, usually one second
FVC	Forced vital capacity, performed with a maximally forced expiratory effort
HAD	Health Assessment Document
IAEA	International Atomic Energy Agency
ICP-ES	Inductively coupled plasma-emission spectroscopy
i.p.	intraperitoneal
i.t.	intratracheal
i.v.	intravenous
LOEL	Lowest observed effect level
MIG	Metal inert gas, a welding process
MMA	Manual metal arc, another welding process
MMAD	Mass median aerodynamic diameter
NAA	Neutron activation analysis
NADB	National Aerometric Data Branch
NADPH	Reduced nicotinamide adenine diphosphate
ND	Not detected
NR	Not reported
NIOSH	National Institute for Occupational Safety and Health
PAM	Pulmonary alveolar macrophage
RDA	Recommended Daily Allowance
SAROAD	Storage and Retrieval of Aerometric Data, replaced in 1987 by AIRS
SRM	Standard reference materials
TIG	Tungsten inert gas, another type of welding
TR-XRF	Total reflection Xray analysis
VC	Vital capacity

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment on the non-carcinogenic effects of chromium to serve as a source document for EPA use. The health assessment was developed for use by the Office of Air Quality Planning and Standards to support decision making regarding possible regulation of chromium as a hazardous air pollutant.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses is placed in perspective with observed environmental levels.

The relevant literature for this document has been reviewed through June, 1986. Selected studies of more recent publications through December, 1989 have been incorporated in the sections on toxicity.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

1. INTERPRETIVE SUMMARY AND CONCLUSIONS

The purpose of this update is to address several technical issues related to noncarcinogenic health effects of chromium compounds that require further clarification. Material previously used for the 1984 document has been reviewed and cited, when appropriate. This update to the 1984 Health Assessment Document (HAD) addresses the following issues:

(1) Oxidation states and persistence of these states in the environment.

(2) Sampling and analytical methodology to differentiate these oxidation states and amounts at submicrogram ambient air levels.

(3) Degree of human exposure to chromium in the environment, both short and long-term.

(4) *In vivo* reduction of Cr (VI) to Cr (III).

(5) Effects from environmentally relevant levels on pulmonary function and on kidney function.

These issues are addressed in this section of the update. The remaining material can be used to supplement this discussion. Approximately 200 new references were reviewed for consideration in this update to the 1984 HAD for chromium, many of which have been published since the completion of the 1984 HAD.

1.1 CHROMIUM OXIDATION STATES AND THEIR PERSISTENCE IN THE ENVIRONMENT

The most chemically stable state for chromium is trivalent chromium [Cr(III)], which comprises most of the total chromium in the environment. Hexavalent chromium [Cr(VI)] is readily reduced to Cr(III) and, in the presence of organic material and particularly at lower pH levels, forms stable Cr(III) complexes. Under certain conditions, Cr(III) will oxidize to Cr(VI). The important variable in this reaction is the presence of manganese oxide, which is

reduced as Cr(III) is oxidized. Cr(III) also is oxidized to Cr(VI) when ore containing Cr(IV) is roasted at high temperatures. The type and amounts of chromium valence states in the ambient environment are not well characterized. The oxidation state of chromium in the ambient air most likely depends on the proximity to sources that emit one form over the other, or mixtures of both. Because Cr(III) is found naturally in the earth's crust, most of the airborne chromium in areas not source-dominated is probably of the trivalent state, but this has not been tested empirically. Additional research is needed to develop quantitative data and mathematical descriptions for predicting the chemical attenuation of chromium in the environment. For now, however, the available data indicate that, in the absence of a nearby source of Cr(VI), chromium exists primarily as Cr(III) in an atmospheric environment that is normally slightly acidic. It appears that Cr(VI) exists primarily in the fine particle phase. This conclusion was reached based on limited data from a few source-specific locations, where Cr(VI) accounted for about 35% of the total mass and 85% of the particle mass smaller than 10 μm.

1.2 SAMPLING AND ANALYTICAL METHODOLOGY FOR EACH OXIDATION STATE AT RELATIVELY LOW LEVELS

Reliable monitoring methods to speciate chromium oxidation states, Cr(III) and Cr(VI), at ambient air levels of less than 1 $\mu g/m^3$ are not available. Several research methods are being developed that may be amenable to routine monitoring of Cr(VI). Some of the more prominent problems with the existing methods include the following:

(1) the presence of other atmospheric contaminants that interfere in the sampling and collection procedure;

(2) losses during sample pretreatment;

(3) oxidation/reduction of the chromium in the sample during analysis;

Some comparative studies presented in the analytical section of this update suggest ways to mitigate some of these problems, but at the expense of accuracy and sensitivity. In general, the methods used routinely to monitor total chromium in ambient air, such as neutron

activation analysis, a nondestructive technique, are accurate and sensitive to relatively low total chromium levels (less than 1 $\mu g/m^3$). Pretreatment of the sample and use of other collection methods to determine oxidation state lack the sensitivity to measure these species at the levels found commonly in ambient air. In conclusion, the speciation of chromium in ambient air cannot be determined with any degree of confidence with currently available instrumentation.

1.3 DEGREE OF EXPOSURE TO CHROMIUM IN THE ENVIRONMENT

Little new information was found on the types of chromium and compounds occurring in the environment. As noted above, analytical methods available for differentiating Cr(III) from Cr(VI) in occupational settings are not sufficiently sensitive or selective for ambient air monitoring. Accordingly, knowledge about the forms of chromium emitted and the transport, transformation, and persistence of these species is the main tool that can be used to estimate the abundance of each oxidation state in specific environments.

The primary sources of emissions of hexavalent chromium appear to be chemical manufacturing and cooling towers. Based on estimated total chromium emissions and percent Cr(VI), these two source categories account for approximately 80% of total Cr(VI) emitted. Theoretically, much of this may be transformed into Cr(III) over a protracted period of time; however, populations close to these sources would be at a greater risk of exposure to Cr(VI) than would the general population.

Manual metal arc (MMA) welding generates three to four times more fumes per kilograms of welded stainless steel than metal inert gas (MIG) welding at the same power; the total chromium contents of MMA welding fumes ranges from 2.4 to 7.0%, 40 to 90% appears in a hexavalent and soluble form. MIG welding fumes may contain 4 to 15% chromium, but most of it is in the trivalent or metallic form.

The most recent data available from EPA's National Air Data Branch (NADB, n.d.) for total chromium show that the highest measured 24-hr chromium level nationwide was 0.6 $\mu g/m^3$, in Camden, NJ. With the use of standard meteorological dispersion factors, the 24-hr reading translates into a 1-hr level of 1.5 $\mu g/m^3$. The maximum annual mean

(arithmetic), also in Camden, for 1984 was 0.08 $\mu g/m^3$. But on average, annual levels nationwide rarely exceed the limit of detection i.e., 0.005 $\mu g/m^3$. The monitoring sites were not located in areas typically dominated by chromium emissions. EPA currently is conducting atmospheric dispersion modeling to improve estimates of exposure to chromium for the general public.

1.4 COMPOUND DISPOSITION AND KINETICS

In the 1984 HAD, the data base for evaluating the *in vivo* reduction of Cr(VI) to Cr(III) was very limited, and no conclusions could be drawn about the importance of such conversions for metabolism and toxicity of Cr(VI) compounds.

In recent years, much work has been performed on *in vivo* reduction, and some valid characterizations now can be made. Many efficient systems for *in vivo* reduction of Cr(VI) exist (Petrilli and De Flora, 1988). The respiratory system, as well as the gastrointestinal tract, provide physiologic environments which have the potential to reduce significant amounts of Cr(VI). Any Cr(VI) that is taken up into the blood may be reduced in the plasma; and if Cr(VI) passes into the red cells, these have a large capacity to reduce Cr(VI) to Cr(III), which will be bound and stored in the cells. Cr(VI) reduction can occur in the cytosol. Ascorbic acid (Vitamin C) is a reducing agent that plays an important protective role against Cr(VI) toxicity in human beings (Korallus, 1986) and experimental animals (Suzuki, 1988; Ginter et al., 1989).

The lack of certain kinetic information about reduction of Cr(VI) to Cr(III) makes it difficult to complete a characterization of the efficiency of Cr(VI) reduction since it will be dependent on the dose and the nature of the reduction environment (e.g., gastric juices have peak reductive capacity 2 to 4 hrs after a meal and reaching a minimum between meals and at night). In general most Cr(VI) would be expected to convert to Cr(III) with absorption and distribution showing much more Cr(III) present than Cr(VI). Cr(VI) is preferentially absorbed, however. Studies to date have not definitively shown that Cr(VI) survives long enough for excretion although kidney toxicity is greater with Cr(VI) exposure than with Cr(III).

1.5 THE ESSENTIALITY OF CHROMIUM

Cr(III) is essential for animals and human beings since it potentiates insulin action in peripheral tissue. Chromium deficiency may cause changes in the metabolism of glucose and lipids. In some studies, dietary supplementation with chromium reverses changes in glucose tolerance and serum lipids. Chromium deficiency is difficult to diagnose since at present no good indicator for tissue-level chromium exists.

1.6 EFFECTS OF CHROMIUM EXPOSURE ON RESPIRATORY AND RENAL SYSTEMS

In much of the literature published since the completion of the 1984 Chromium HAD, together with earlier studies, qualitative information on the association between respiratory tract irritation and Cr(VI) exposure was reported. Few of the available studies, however, provide quantitative concentration-response data on chromium health effects.

Three studies on chromeplaters seem to provide some quantitative information on upper respiratory irritation after exposure to Cr(VI) as chromic acid. In the study of Cohen et al. (1974), nasal ulcers and perforations were associated with total chromium concentrations of 1.4 to 43.9 $\mu g/m^3$, averaging 7.1 $\mu g/m^3$, and Cr(VI) concentrations of 0.09 to 9.1 $\mu g/m^3$, averaging 2.9 $\mu g/m^3$. Ninety-five percent of the 37 workers studied exhibited pathologic changes in nasal mucosa and in a concentration-duration response. More than half of the workers employed less than one year had nasal pathology that was more severe than simple redness of the nasal mucosa. Almost all the workers (35 of 37) employed longer than one year had nasal tissue damage. The authors noted the lack of good industrial hygiene practices, implicating direct contact, such as touching of the nose with chromium-contaminated hands, as a potentially important route of exposure. A subsequent study by Lucas and Kramkowski (1975) revealed similar results. Cr(VI) concentrations ranged from 1 to 20 $\mu g/m^3$, averaging 4 $\mu g/m^3$. However, the authors attributed the nasal pathology primarily to direct contact. Lindberg and Hedenstierna (1983) also found similar effects on nasal pathology and subjective symptoms. They reported reddening of the nasal mucosa at 1 to 2 $\mu g/m^3$ and nasal irritation (chronic and nasal septal ulceration and perforation) in two-thirds of the subjects at concentrations from 2 to 20 $\mu g/m^3$. All workers with nasal ulceration

had been exposed to chromic acid mist, which contained Cr(VI) at 20 $\mu g/m^3$ or greater near the baths. For pulmonary function measurements, changes in vital capacity and forced expiratory volume at one sec (FEV_1) were seen from Cr(VI) exposures greater than 2 $\mu g/m^3$.

Cr(VI) exposure as low as 4 to 6 $\mu g/m^3$ can result in renal effects. Elevated urinary excretion of β_2-microglobulin is an indicator of nephrotoxicity. Lindberg and Vesterberg (1983b) observed increased elimination of this protein in chromeplaters exposed to 8-hr shifts of 4 to 20 $\mu g/m^3$ Cr(VI). The effect is probably reversible since former chromeplaters did not have an elevated concentration of either β_2-microglobulin or albumin in their urine.

In another study, Saner et al. (1984) did not find increased urinary β_2-microglobulin levels in tannery workers in comparison to referent control workers. However, comparison of urinary chromium concentrations of the tannery workers in this study versus the chromeplaters in the Lindberg and Vesterberg (1983a,b) study suggests that the latter had distinctly higher chromium exposures than the former.

Hexavalent chromium compounds may cause skin lesions by direct contact, but exposure to such compounds can also cause sensitization, which may lead to dermatitis and eczema. Chromium allergy is mainly an occupational problem and not common in the general population.

In conclusion, transient and subtle effects on the airways and renal effects have been observed in chromeplaters exposed subchronically to chromic acid mist containing Cr(VI) in air at concentrations greater than 1 $\mu g/m^3$. Such effects include: reddening of nasal mucosa starting at 1 to 2 $\mu g/m^3$; nasal irritation (ulceration, perforation) at 2 to 20 $\mu g/m^3$, changes in pulmonary function (FEV_1) at levels >2 $\mu g/m^3$; and renal proteinuria at 2 to 20 $\mu g/m^3$. The 1 $\mu g/m^3$ level, therefore, appears to represent the lowest observed-effect level (LOEL) associated with exposure to chromic acid. It is difficult to specify whether 1 $\mu g/m^3$ should also be identified as the lowest observed adverse effects (LOAEL) level. The observed effects between 1 and 2 $\mu g/m^3$ may not constitute functional impairment of human activity, but could be an early indicator of altered normal functioning that might lead to a progression to more serious health effects if long-term sustained exposure occurred at this level. However, the data by Lindberg and Hedenstierna (1983) did not indicate any chronic effects. The data on other chromium compounds do not clearly identify a LOEL.

Chromic acid represents the worst case since it is highly soluble and reactive. By applying an uncertainty factor of 10 because LOAELs are used rather than NOAELs (no-observed-adverse-effect-levels), exposure to 0.1 μg Cr(VI)/m^3 should not cause irritation of the airways or other local or systemic effects. Cr(VI) will be reduced to Cr(III) in the lungs, and that chromium species will be retained in the lungs, showing a tendency to increase with age.

2. INTRODUCTION

In August 1984, the U.S. EPA's Environmental Criteria and Assessment Office (ECAO) completed an in-depth review of the scientific literature on chromium and its compounds for the Office of Air Quality Planning and Standards. Published as the Health Assessment Document (HAD) for chromium (U.S. Environmental Protection Agency, 1984a), it was the scientific data base to be used for regulatory decision making by the Agency and as an interpretive summary of all relevant scientific studies. The HAD considered all sources of chromium in the environment, the likelihood for human exposure, and the possible consequences to man and lower organisms from its absorption. The information was integrated into a format that could serve as the basis for qualitative risk assessments; at the same time, it identified gaps in scientific knowledge that limited accurate health assessment.

Notwithstanding the in-depth analysis, peer-review processes, and multiple revisions of the 1984 Chromium HAD, several salient scientific questions concerning noncarcinogenic effects remained unanswered. To address those issues, a new literature review was initiated in selected areas, key studies were reanalyzed, and some of the conclusions of the original HAD were reassessed. Approximately 200 additional references were reviewed for possible inclusion in the revised HAD. Although this additional material and the reanalysis of previously reviewed data add considerably to understanding the effect of chromium on human health, many of the questions still are not answered completely, though the confidence in the evaluation has increased markedly. In this update the following technical issues have been addressed:

- Types and persistence of chromium compounds in the environment
- Adequacy of the sampling and analytical methods as a means of evaluating the types and amounts of chromium in environmental and controlled study exposures
- Transformation rates of chromium compounds in the environment
- Exposure parameters associated with the key studies
- In-depth review of pulmonary effects

- Concentration-response relationships of acute, subchronic, and chronic respiratory effects, including chemical and physical properties of the active chromium species that influence deposition, absorption, and other pharmacokinetics.

In this update, all key references cited in the 1984 document were reviewed again and compared with their descriptions in the HAD. Sometimes no changes were made; in some instances, the original descriptions were revised.

Several other assessments of health effects of trivalent and hexavalent chromium have been prepared by EPA (U.S. Environmental Protection Agency, 1984b,c; Syracuse Research Corporation), World Health Organization (1988) and the ATSDR Tox Profile on Chromium (Syracuse Research Corporation, 1989) are available. Carcinogenic potency of different valence states of chromium is also under consideration (U.S. Environmental Protection Agency, 1987). In addition, there is a recent summary chapter (Hertel, 1986) which is useful for review purposes.

3. BACKGROUND INFORMATION

3.1 CHEMICAL AND PHYSICAL PROPERTIES

Chromium is one of the most important metals used in industry today. Discovered in 1797 by the French chemist Louis Vanquelin, chromium was a key ingredient in the industrial revolution. Table 3-1 lists its chemical and physical properties.

Although chromium exists in several oxidation states, from -2 to +6, chromium +3 and +6 [Cr(III) and Cr(VI)] have been studied extensively, and the other species have been investigated only moderately in research chemistry. The action of these two forms on biological systems is characterized poorly. The intermediate oxidation states of chromium, +4 and +5, also may have an important role in interactions with biological systems, but until recently, virtually no biological research had been conducted on these chemical species.

Cr(III) is the most stable form of chromium. In neutral and basic solutions, Cr(III) forms binuclear and polynuclear compounds in which adjacent chromium atoms are linked through hydroxy- (OH) or oxo- (O) bridges. Interestingly, Cr(III) forms stable complexes with amino acids and peptides. Cr(III) also has a strong tendency to form hexacoordinated octahedral complexes with ligands, such as water, ammonia, urea, ethylenediamine, halides, sulfates, or organic acids. These relatively stable complex formations (Cotton and Wilkinson, 1980; Kiilunen et al., 1983) can prevent precipitation of Cr(III) at pH values at which it would otherwise precipitate, and at normal pH values further oxidation of Cr(III) is unlikely (Hartford, 1986).

Cr(VI) exists in solution as hydrochromate, chromate, and dichromate ionic species. The proportion of each ion in solution is dependent both on pH and on concentration (Pourbaix, 1974). In strongly basic and neutral pH values, the chromate form predominates. As the pH is lowered, the hydrochromate concentration increases. At very low pH, the dichromate species predominates. In the pH ranges encountered in natural water, the predominant forms are hydrochromate ions (63.6%) at pH 6.0 to 6.2 and chromate ions (95.7%) at pH 7.8 to 8.5. The oxidizing ability of Cr(VI) in aqueous solution is pH-dependent. The oxidation potential of Cr(VI) increases at lower pH. The ability of Cr(VI) to oxidize organic materials and the tendency of the resulting Cr(III) to form stable complexes

TABLE 3-1. CHEMICAL AND PHYSICAL PROPERTIES OF CHROMIUM

Property	Value
Atomic weight	51.996
Isotopes, %	
50	4.31
52	83.76
53	9.55
54	2.38
Crystal structure	body-centered cube
Density at 20°C, g/cm^3	7.19
Melting point, °C	1875
Boiling point, °C	2680
Vapor pressure 130 P[a]ab, °C	1610
Heat of fusion, kJ/mol	13.4-14.6
Latent heat of vaporization at bp kJ/mol[b]	320.6
Specific heat at 25°C, kJ/(mol-K)[b]	23.9 (0.46 kJ/kg-K)
Linear coefficient of thermal expansion at 20°C	6.2×10^{-6}
Thermal conductivity at 20°C, W/(m-K)	91
Electrical resistivity at 20°C, μ-m	0.129
Specific magnetic susceptibility at 20°C	3.6×10^{-6}
Total emissivity at 100°C, nonoxidizing atm, in %	0.08
Reflectivity, R	
λ, nm	300, 500, 1,000, 4,000
%	67, 70, 63, 88
Refractive index	
α	1.64-3.28
λ	2570-6080
Standard electrode potential, valence 0 to 3+, V	0.71
Ionization potential, V	
1st	6.74
2nd	16.6
Half-life of ^{51}Cr isotope, days	27.8
Thermal neutron scattering cross section, m^2	6.1×10^{-28}
Elastic modulus, GPa[c]	250
Compressibility, at 10-60 TPa[d]	70×10^{-3}

[a]To convert Pa to mmHg, multiply by 0.0075.
[b]To convert J to cal, divide by 4.184.
[c]To convert GPa to psi, multiply by 145,000.
[d]99% Cr; to convert TPa to megabars, multiply by 10.

Source: Radian Corporation (1984).

with available biological ligands afford a reasonable mechanism by which chromium can interact with the normal biochemistry of man.

The solubility of a specific chromium compound can be an important factor in determining its health effects from welding fumes and similar mixture of chromium particles. Studies of water solubility (determined by shaking 30 minutes at room temperature) have shown that, in welding fumes, only Cr(VI) compounds are water soluble, and some of these only slightly so (Stern, 1982). Some Cr(VI) and all Cr(III) and Cr(O) compounds are not water soluble.

The physical properties of various chromium oxidation states and of several environmentally significant trivalent and hexavalent chromium compounds are shown in Table 3-2. Because of the considerable disagreement in the literature concerning the physical parameters given in this table, these values should be accepted with reservation. The disagreement in the values is possibly due to the reactions of these compounds with other substances, namely the moisture and air at high temperatures, impurities, and structural and compositional changes occurring during the experimental determinations. The composition of typical ferrochromium alloys and chromium metals is given in Table 3-3. General information on the chemistry of chromium can be found in the 1984 HAD (U.S. Environmental Protection Agency, 1984a).

3.2 PRODUCTION, USE, AND RELEASE TO THE ENVIRONMENT

Considerable information is available on production, use, and release of chromium into the environment. Much less information is available on the forms of chromium in the environment. Although it is assumed that Cr(III) and Cr(VI) comprise most of the total environmental chromium, the biological importance of the other oxidation states cannot be ruled out completely.

This section focuses mainly on new information not presented in the 1984 HAD.

3.2.1 Production of Chromium Compounds

According to the U.S EPA's 1984 report (Radian Corporation, 1984) on chromium emission factors, chromite ore has not been mined commercially in the United States since

TABLE 3-2. OXIDATION STATES OF SELECTED CHROMIUM COMPOUNDS AND THEIR MAJOR PHYSICAL PROPERTIES

Compound by Oxidation State	Formula	Density, g/cm^3	Melting Point, °C	Boiling Point, °C	Solubility g/100 ml
Oxidation State 0					
Chromium carbonyl	$Cr(CO)_6$	1.77	150 (decomposes) (sealed tube)	151 (decomposes)	Slightly soluble in CCl_4; insoluble in H_2O, $(C_2H_5)_2O$, C_2H_3OH, and C_6N_6
Dibenzene-Chromium(0)	$(C_6H_6)_2Cr$	1.519	284-285	Sublimes at 150 (vacuum)	Insoluble in H_2O; soluble in C_6H_6
Oxidation State +1					
bis(bipheny1)-chromium (I) iodide	$(C_6H_5C_6H_5)_2CrI$	1.617	178	Decomposes	Soluble in C_2H_5OH, C_5H_3N
Oxidation State +2					
Chromous acetate	$(Cr_2(C_2H_3O_2)_4 \cdot 2H_2O$	1.79			Slightly soluble in H_2O; soluble in acid
Chromous chloride	$CrCl_2$	2.93	815	1,120	Soluble in H_2O to blue solution, absorbs O_2
Chromous ammonium sulfate	$CrSO_4(NH_4)_2SO_4 \cdot 6H_2O$				Soluble in H_2O, absorbs O_2

TABLE 3-2 (cont'd). OXIDATION STATES OF SELECTED CHROMIUM COMPOUNDS AND THEIR MAJOR PHYSICAL PROPERTIES

Compound by Oxidation State	Formula	Density, g/cm^3	Melting Point, °C	Boiling Point, °C	Solubility g/100 ml
Oxidation State +3					
Chromic acetate	$Cr(CH_3COO)_3 \bullet H_2O$	NR	NR	NR	Slightly soluble
Chromic chloride	$CrCl_3$	2.76	1,150	1,300 (sublimes)	Insoluble
Chromic chloride, hexahydrate	$(Cr(H_2O)_4Cl_2)Cl \bullet 2H_2O$	1.76	83	NR	58.5 at 25°C
	$(Cr(H_2O)_6)Cl_3$	NR	NR	NR	Soluble
Chromic formate, hexahydrate	$(Cr(HCOO)_3) \bullet 6H_20$	NR	decomposes above 300	NR	Soluble
Chromic oxide	Cr_2O_3	5.21	2,266	4,000	Insoluble
Chromic phosphate	$CrP0_4 \bullet 2H_2O$	2.42 (32.5°C)	NR	NR	Slightly soluble
Hydrated chromic phosphate	$CrPO_4 \bullet 6H_2O$	2.121 (14°C)	100	NR	Insoluble
Chromic sulfate	$Cr_2(SO_4)_3$	3.012	NR	NR	Insoluble
Chromic sulfate	$Cr_2(SO_4)_3 \bullet 15H_2O$	1.867 (17°C)	100	100(-100 H_2O)	Soluble
Hydrated chromic sulfate	$Cr_2(SO_4)_3 \bullet 18H_2O$	1.7 (22°C)	100 (-10H_2O)	NR	120 at 20°C

TABLE 3-2 (cont'd). OXIDATION STATES OF SELECTED CHROMIUM COMPOUNDS AND THEIR MAJOR PHYSICAL PROPERTIES

Compound by Oxidation State	Formula	Density, g/cm^3	Melting Point, °C	Boiling Point, °C	Solubility g/100 ml
Oxidation State +4					
Chromium(IV) oxide	CrO_2 Dark brown or black powder	4.98 (calculated)	Decomposes to Cr_2O_3		Soluble in acids to Cr^3+ and Cr^6+
Chromium (IV) chloride	$CrCl_4$		830		
Oxidation State +5					
Barium chromate (V)	$Ba_3(CrO_4)_2$ Black-green crystals			Slightly decomposes in H_2O dilute acids to Cr_3+ and Cr_6+	Soluble in dilute acids to Cr^3+ and Cr^6+
Oxidation State +6					
Ammonium chromate	$(NH_4)_2\ CrO_4$	1.91^{12}	180 decomposes	NR	40.5 at 30°C
Ammonium dichromate	$(NH_4)_2Cr_2O_7$	2.155^{25}	180 decomposes	NR	30.8 at 15°C
Barium chromate	$BaCrO_4$	4.498^{25}	decomposes	NR	3.4×10^{-4} at 160°C
Chromium (VI) oxide	CrO_3	2.70^{25}	197	Decomposes	67.45 at 100°C
Lead chromate	$PbCrO_4$	6.12^{15}	844	Decomposes	5.8×10^{-6} at 25°C

TABLE 3-2 (cont'd). OXIDATION STATES OF SELECTED CHROMIUM COMPOUNDS AND THEIR MAJOR PHYSICAL PROPERTIES

Compound by Oxidation State	Formula	Density, g/cm^3	Melting Point, °C	Boiling Point, °C	Solubility g/100 ml
Oxidation State +6 (Cont.)					
Mercurous (I) chromate	Hg_2CrO_4	NR		NR	very slightly soluble
Mercuric (II) chromate	$HgCrO_4$	NR	decomposes	NR	slightly soluble, decomposes
Potassium chromate	K_2CrO_4	2.732^{18}	971	NR	62.9 at 20°C
Potassium dichromate	$K_2Cr_2O_7$	2.676^{25}	398	500 decomposes	4.9 at 0°C 102 at 100°C
Sodium chromate	Na_2CrO_4	2.723^{25}	792	NR	87.3 at 30°C
Sodium dichromate dihydrate	$Na_2Cr_2O_7 \cdot 2H_2O$	2.348^{25}	84.6 (incongruent)	400 decomposes	180 at 20°C

[a]NR = not reported.

Source: Stern (1982).

TABLE 3-3. COMPOSITION OF TYPICAL FERROCHROMIUM ALLOYS AND CHROMIUM METAL (PERCENT)

Grade	Chromium	Silicon	Carbon	Sulfur[a]	Phosphorus[a]	Other[b]
Ferrochromium						
High-carbon	66-70	1-2	5-6.5	0.04	0.03	
High-carbon, high-silicon						
blocking chrome	55-63	8-12	4-6	0.03		
exothermic ferrochrome	41-51	9-14	3.6-6.4	0.03		
foundry ferrochrome	55-63	8-12	4-6			
Refined chrome	53-63	2.5[a]	3-5	0.03		
SM ferrochrome	60-65	4-6	4-6			4-6 manganese
Charge chromium						
50-55 percent chromium	50-56	3-6	6-8	0.04	0.03	
66-70 percent chromium	66-70	3	6-6.5	0.04	0.03	
Low-carbon						
0.025 percent carbon	67-75	1[b]	0.025[a]	0.025	0.03	
0.05 percent carbon	67-75	1[b]	0.05[a]	0.025	0.03	
Simplex	63-71	2.0[a]	0.01 or 0.025			
Ferrochromium-silicon:						
36/40 grade	35-37	39-41	0.05[a]			
40/43 grade	39-41	42-45	0.05[a]			
Chromium metal						
Electrolytic	99.3[c]	0.01[a]	0.02[a]	0.03		0.5 oxygen[a] 0.05 nitrogen[a]
Aluminothermic	99.3[c]	0.15[a]	0.05[a]	0.015	0.01	0.2 oxygen[a] 0.3 aluminum[a]

[a]Maximum value.

[b]Difference between sum of percentages shown and 100 percent is chiefly iron content.

[c]Minimum value.

Source: Radian Corporation (1984).

1961, when the U.S. Defense Production Act was phased out, eliminating government subsidization of chromite mining activities. The United States owns chromite deposits in Maryland, Montana, North Carolina, California, Wyoming, Washington, Oregon, Texas, and Pennsylvania; however, the low chromium content of these deposits precludes economical mining. In 1982, the U.S. imported 456,000 metric tons (507,000 tons) of chromite, mostly from South Africa (54.6 percent), the Phillipines (13.8 percent), Finland (8.9 percent), Madagascar (8.1 percent), the U.S.S.R (6.7 percent), Turkey (6.3 percent), Albania (0.8 percent), and Pakistan (0.6 percent).

In 1984, the U.S. annual production capacity of sodium chromate and sodium dichromate was 204,000 metric tons. Chromic acid annual production capacity totaled 38,000 metric tons (Myers et al., 1986). The industrial processes for the production of chromium metal and compounds were described adequately in the previous document (U.S. Environmental Protection Agency, 1984a).

3.2.2 Uses of Chromium and Its Compounds

The 1984 Chromium HAD (U.S. Environmental Protection Agency, 1984a) noted that metallurgical and chemical usages constituted 82% of the total U.S. chromium consumption in 1979. The major chromium chemicals, uses, and the number of production sites are presented in Tables 3-4 and 3-5. As noted in the 1984 Chromium HAD, the pattern of chromium consumption in the United States has been consistent over the last 20 years. However, the use of chromite and chrome alloys in the refractory industry is beginning to decline as open-hearth furnaces are replaced by basic-oxygen furnaces. In the future, growth in chromium usage is expected in the metallurgical and chemical sectors.

3.2.3 Releases Into the Environment

As seen in Table 3-6, comfort cooling towers account for the largest number of sources emitting Cr(VI) compounds. With an estimated 38,000 sources, the estimated 7.2 to 206 metric tons per year amounts to a relatively significant contribution of the total hexavalent chromium emissions, but on a site-by-site basis, most of the individual towers do not appear to be significant sources of hexavalent chromium emissions, averaging a maximum of 0.005 metric tons per year. Although the combustion of coal and oil represents the largest

TABLE 3-4. MAJOR CHROMIUM USES AND KEY CHROMIUM CHEMICALS INVOLVED

Chromium Chemical Use Area	Key Chromium Chemicals Involved
Paints and Pigments	Chrome Yellow[a] Chrome Orange[a] Chrome Oxide Green Molybdate Orange[a] Chrome Green
Leather Tanning Liquor	Basic Chromium Sulfate
Metal Finishing and Plating	Chromic Acid
Corrosion Inhibitors	Zinc Chromate Zinc Tetroxychromate Strontium Chromate Lithium Chromate
Catalysts	Cadmium Chromate Copper Chromate Magnesium Dichromate Nickel Chromate Copper Chromite
Drilling Muds	Chromium Lignosulfonate
Wood Preservatives	Chrome Copper Arsenate Chrome Zinc Chloride
Textile Mordants and Dyes	Chromic Chromate Chromic Chloride (hydrated) Chromic Fluoride Chromic Lactate

[a]Contains lead chromate.

Source: Radian Corporation (1984).

TABLE 3-5. LIST OF COMMERCIALLY PRODUCED SECONDARY CHROMIUM CHEMICALS AND THEIR GENERAL USES

Chromium Chemical[a]	Number of Production Sites[b]	General Uses
Chromic acid (Chromium trioxide)	2	Electroplating
Chromium acetate	6	Printing and dyeing textiles
Chromium acetylacetonate	3	Catalysts, antiknock compounds
Chromium monoboride	1	Unknown
Chromium carbide	1	Metallurgy
Chromium carbonyl	2	Catalysts
Chromium chloride, basic	1	Metal treatment
Chromium chloride	2	Metal treatment
Chromium diboride	1	Unknown
Chromium difluoride	1	Catalysts
Chromium dioxide	1	Magnetic tape
Chromium 2-ethylexanoate (Chromic octoate)	2	Unknown
Chromium fluoride	1	Mordants, catalysts
Chromium hydroxide	1	Pigments, catalysts
Chromium hydroxy diacetate	1	Unknown
Chromium hydroxy dichloride	1	Unknown
Chromium naphthenate	2	Textile preservative
Chromium nitrate	2	Catalysts, corrosion control
Chromium oleate	2	Unknown
Chromium oxide (Chrome oxide green)	6	Pigments
Chromium phosphate	2	Pigments, catalysts
Chromium potassium sulfate (Chrome alum)	1	Photographic emulsions
Chromium sulfate	2	Catalysts, dyeing, tanning
Chromium sulfate, basic	1	Tanning
Chromium triacetate	1	Unknown
Chromium trifluoride	1	Printing, dyeing, catalysts
Chrome lignosulfate	1	Drilling muds
Potassium chromate	1	Metal treatment
Potassium dichromate	1	Tanning, dyeing, pigments
Lead chromate	5	Pigments
Zinc chromate	3	Corrosion control
Ammonium dichromate	2	Printing, pyrotechnics
Barium chromate	2	Pyrotechnics
Calcium chromate	3	Corrosion control
Cesium chromate	1	Electronics
Copper chromate, basic	1	Wood preservative
Magnesium chromate	1	Refractory, catalysts
Strontium chromate	3	Corrosion control pigment
Iron chromite	2	Refractory

[a]List does not include sodium chromate and sodium dichromate, which are primary chemicals.
[b]Several sites produce multiple chromium chemicals.

Source: Radian Corporation (1984).

TABLE 3-6. SOURCES AND ESTIMATES OF UNITED STATES ATMOSPHERIC CHROMIUM EMISSIONS

Source Category	Estimated Number of Sources	Chromium Emissions (Metric Tons/Yr)	Estimated Cr(VI) (%)
Combustion of Coal and Oil	Many	1,723	0.2
Chromium Chemical Manufacturing	2	18	67
Chemical Manufacturing Cooling Towers	2,039	43	100
Petroleum Refining Cooling Towers	475	32	100
Specialty/Steel Production	18	103	2.2
Primary Metal Cooling Towers	224	8	100
Chromeplating	4,000	700	~100
Comfort Cooling Towers	38,000	7.2-206	100
Textile Manufacturing Cooling Towers	51	0.1	100
Refractory Production	10	24	1.3
Ferrochromium Production	2	16	5.4
Sewage Sludge Incineration	133	13	<0.1
Tobacco Cooling Towers	16	0.2	100
Utility Industry Cooling Towers	6	1.0	100
Chrome Ore Refining	6	4.8	<0.1
Tire and Rubber Cooling Towers	40	0.2	100
Glass Manufacturing Cooling Towers	3	0.01	100
Cement Production	145	3	0.2
Municipal Refuse Incineration	95	2.5	0.3
NATIONWIDE TOTAL		2,700-2,900	

Sources: Myers et al. (1986); Nelson et al. (1984); Radian Corporation (1984); U.S. Environmental Protection Agency (1987).

source category of total chromium emissions, only about 0.2% of these emissions are hexavalent chromium, or 5.4 metric tons Cr(VI) annually. Chemical manufacturing, petroleum-refining cooling towers, primary metal, and chromeplating represent the major Cr(VI) source categories. However, the maximum average of each individual source is less imposing; for instance, the emission average for petroleum-refining cooling towers is only 0.4 metric tons per year.

3.3 ENVIRONMENTAL FATE, TRANSPORT, AND CONCENTRATIONS

3.3.1 Air

Chromium occurs in the atmosphere primarily in two oxidation states, Cr(III) and Cr(VI). The forms and uses are shown in the previous tables. In the environment under typical atmospheric conditions, as theorized by Seigneur (1986) and others, Cr(VI) may be reduced to Cr(III) at a significant rate by vanadium (V2+, V3+, and VO2+), Fe2+, HSO_3^- and As(III). Conversely, the oxidation of Cr(III) to Cr(VI) may occur in the atmosphere at a significant rate only if (1) Cr(III) is emitted as a chromium salt and not Cr_2O_3 and (2) at least 1% of Mn in atmospheric aerosols is present as MnO_2. The time required for these reactions to occur in the environment, given all the other species present, is unknown. Butler et al. (1986) and others reported that chromium occurred in the smaller particle size fractions. Table 3-7 shows the combined results from two kilns at a chemical plant for a total of six impactor measurements. The mean aerodynamic diameter particle size classes are: greater than 10 μm, 2 to 10 μm, and less than 2 μm. Eighty-five percent of the total Cr(VI) was contained in the two smaller size classes, which contained only 35% of the total mass. Similar data were reported by Cox et al. (1985), who determined by scanning electron microscopy that the submicron particles and aggregates of the particles contained the most of the hexavalent chromium.

In general, 24-hr ambient air chromium levels, at monitoring sites not necessarily located near chromium emissions, rarely exceed 0.1 $\mu g/m^3$. In EPA's National Aerometric Data Branch (NADB, n.d.) inventory of daily chromium monitoring, only 8 observations at 173 sites exceeded 0.1 $\mu g/m^3$ as a 24-hr average in 1984. Table 3-8 lists the number of

TABLE 3-7. CHEMICAL PLANT PARTICLE SIZE RESULTS

Size fraction mm	Particulate Mass		Cr(VI) extracted		Cr(III) extracted	
	mg	% of total	μg	% of total chromium	μg	% of total chromium
>10	39.9	62	84.2	15	349	23
2-10	6.6	10	197.8	35	511	35
<2	18.4	28	286.1	50	621	42
Total	64.9	100	568.1	100	1,481	100

Source: Butler et al. (1986).

observations exceeding 0.1 $\mu g/m^3$ from 1977 through 1984. In fact, only about 50 24-hr observations out of the entire data set have exceeded 0.3 $\mu g/m^3$ chromium from 1977 to 1984. Table 3-9 shows the 26 sites at which those 50 observations occurred. Table 3-10, containing the most recent information available from EPA's NADB, lists 24-hr values for total chromium, measured by neutron activation analysis, for the 13 highest sites, taken from an examination of 173 site records for the year 1984. From these sites, which make up the nationwide network, the highest observed 24-hr total chromium concentration was 0.6 $\mu g/m^3$ (in Camden, NJ). Additionally, chromium emissions from only 7 of the 173 sites exceeded 0.1 $\mu g/m^3$, but these monitors generally are not located near sources that emit significant quantities of chromium (Myers et al., 1986).

3.3.2 Soil

Bartlett (1986) investigated the chemistry of chromium in soils. He found that the key parameter for oxidizing Cr(III) to Cr(VI) was fresh manganese oxide, which becomes reduced as the Cr(III) is oxidized. According to Bartlett, this phenomenon has not been reported previously because dried, stored, lab-dirt samples had been studied. In such samples, reducing organics are released, and manganese oxides are reduced or occluded temporarily.

TABLE 3-8. NUMBER OF NADB OBSERVATIONS EXCEEDING 0.1 $\mu g/m^3$ TOTAL CHROMIUM ACCORDING TO YEAR[a]

Number	1977	1978	1979	1980	1981	1982	1983	1984
	28	21	19	17	18	17	29	8

[a]NADB Chromium Inventory from 1977-1984; total of 2,106 possible yearly maxima.

Bartlett noted that the federal toxicity test using acetic acid eliminates the possibility of finding Cr(VI) in most soils.

Whether or not Cr(III), naturally present in soil or added to it, is oxidized depends upon the interaction between the chemical forms of the chromium and the manganese oxides. If the Cr(III) is "moderately available," the regulating factor appears to be the "freshness" of the manganese oxide surfaces, which is related to quantities of oxidizable organic substances and to soil temperature, moisture, aeration, and drying. Strongly bound Cr(III) may remain reduced in soils, although small amounts may be oxidized. Organic forms are more easily oxidized than insoluble oxides. Reduction of Cr(VI) added to soils occurs readily if the soil pH is low and an organic energy source is available. Because soils are not equilibrium systems, reduction of Cr(VI) and oxidation of Cr(III) may occur at the same time in the same soil sample.

TABLE 3-9. NADB SITES EXCEEDING 0.3 $\mu g/m^3$ TOTAL CHROMIUM FROM 1977 TO 1983

Site	Year	No. of samples	Maximum observation $\mu g/m^3$	Arithmetic mean $\mu g/m^3$
Steubenville, OH	1977	21	2.0550	0.5251[a]
	1979	28	0.6839	0.1212[a]
East Chicago, IL	1977	24	1.0750	0.1170
Pasadena, CA	1977	32	0.5600	0.0400[a]
Clarion Co., PA	1977	25	0.4052	0.1475[a]
Greenville, SC	1977	27	0.4031	0.0311
Columbia, SC	1977	11	0.3045	0.0360[a]
Huntington, WV	1977	6	0.3742	0.0885[a]
Torrance, CA	1977	29	0.3153	0.0306
Niagara Falls, NY	1979	30	0.5590	0.0389
Baltimore, MD	1979	26	0.4589	0.0935
	1980	6	0.5794	0.2264
	1982	19	0.4310	0.1019
	1983	23	0.4466	0.0854
Cincinnati, OH	1979	28	0.4316	0.0451
Abilene, TX	1980	53	0.9100	0.0400
Camden, NJ	1980	19	0.4037	0.0903
	1981	30	0.3461	0.0603
New Orleans, LA	1981	30	1.0710	0.0436
Corpus Christi, TX	1981	33	0.7300	0.1200
(2 locations)	1981	36	0.3500	0.0700
Brownsville, TX	1981	51	0.3900	0.0300
Wichita, KS	1982	58	0.3500	0.0150
	1983	56	0.4000	0.0420
Kansas City, KS	1983	42	0.4400	0.0320
Shawnee, KS	1983	57	0.3900	0.0260

[a]Value derived from data that did not meet SAROAD criteria, i.e., at least five valid 24-hr measurements per quarter or 9 of 12 monthly composites.

Source: Derived from NADB data files (n.d.).

TABLE 3-10. HIGHEST MEASURED TOTAL CHROMIUM CONCENTRATIONS FOR THE YEAR 1984, $\mu g/m^3$

Site	Maximum observation	2nd maximum	Arithmetic mean	Geometric mean
Camden, NJ	0.6017	0.2190	0.0834	0.0249
Reading, PA	0.3530	0.1466	0.0618	0.0369
Dundalk, MD	0.3442	0.1386	0.0497	0.0278
Baltimore, MD (1st site: Fire Department)	0.3197	0.2271	0.0626	0.0236
Youngstown, OH	0.1649	0.0163	0.0181	0.0085
St. Louis Park, MN	0.1594	0.0318	0.0114	0.0064
Columbus, GA	0.1502	0.0052[a]	0.0184	0.0071
Cleveland, OH (2nd site: Broadway Avenue)	0.1183	0.1053	0.0332	0.0221
Erie, PA	0.0993	0.0466	0.0161	0.0096
Philadelphia, PA (2nd site: Edgemont and Auburn Streets)	0.0839	0.0428	0.0188	0.0108
Milwaukee, WI (1st site: Greenfield Avenue)	0.0767	0.0416	0.0149	0.0103
Huntington, WV	0.0717	0.0220	0.0128	0.0075
Chattanooga, TN (2nd site: East 11th Street)	0.0713	0.0200	0.0134	0.0082

[a]Apparent error in the data analysis.

Source: Calculated from the files of EPA's National Aerometric Data Branch (NADB, n.d.).

4. QUANTITATIVE ANALYSIS OF CHROMIUM IN BIOLOGICAL AND ENVIRONMENTAL MEDIA

Chromium analysis poses a number of problems in the acquisition of dose-effect and dose-response relationships for this element. First, levels of the element in biological media are at the trace and ultra-trace level, even under conditions of occupational, environmental, or accidental exposure. Secondly, chromium's toxicological and biological behavior is closely linked to its chemical form, notably the valency state. Thirdly, environmental transformations in the valency of this element can occur after dispersal as can artifactual changes of oxidation state during sampling and analysis.

This chapter update for chromium analysis has two major sections: total chromium analysis in biological and environmental media and chemical speciation measurements in various biological and environmental media.

4.1 TOTAL CHROMIUM ANALYSIS IN BIOLOGICAL AND ENVIRONMENTAL MEDIA

In many cases, it is sufficient to simply measure the total chromium content of various media while in other circumstances it may not be readily feasible to do chemical form speciation. In the analysis of human physiological fluids, for example, the biochemical milieu and the presence of variably bound chromium species rules out methodologies which permit simple partitioning of valence state in monitoring of general populations.

4.1.1 Biological Media Sampling Steps in Total Cr Measurements

In total chromium analyses, preservation of specific chemical species of chromium is not a compelling problem, and precautions to be taken are basically those for most elements at the ultra-trace level in biological and environmental media. Measurements of total chromium in biological media entail potential hazards of sample contamination, sample loss, and other variables which affect precision and accuracy.

Sample Collecting Steps

Specifics of the sample collection method are determined by the nature of the testing matrix itself. Since media background levels are at the picogram and subnanogram levels, extremely scrupulous care to avoid sample contamination must be taken. It is strongly recommended at the outset that only well-trained personnel with experience in ultra-trace analysis of elements and associated field work be employed for total chromium analysis (Versieck et al., 1982; Behne, 1981). Sample collection steps are summarized in Table 4-1. Ordinary blood collection needles can contaminate blood samples by 2 to 3 orders of magnitude because of their leachable chromium content. Similarly use of surgical steel blades for soft tissue sampling must be avoided (Versieck et al., 1982).

Various sample collection alternatives which avoid contamination are available. Versieck et al. (1979) used a plastic polypropylene over-the-needle catheter while Halls and Fell (1981) used a plastic cannula to collect whole blood. All-plastic syringes with silanized needles in a butterfly-type configuration are reported to be satisfactory (Veillon et al., 1984).

Similarly, collection containers can provide opportunities for both contamination as well as sample loss – the latter by transfer of chromium from bulk matrix to the vessel wall. Fell et al. (1980) advise against use of any containers which have not been thoroughly evaluated for Cr release. Chromium levels in serum are so low that even conventional acid washing of blood tubes is inadequate. Extended boiling in Cr-free acids and surface silanization of high-purity quartz tubes are recommended for serum collection and processing (Veillon, 1988). Spot urine samples (ca. 25 ml) are collected best in rigorously acid-washed plastic containers. Similarly, 24-h collections should employ 2-L polypropylene plastic bottles washed and rinsed in low-chromium acids and chromium-free deionized water.

Collection procedures similar to that for biological media are required for environmental samples such as natural waters, while grossly contaminated samples, such as from Superfund waste sites, may allow somewhat less stringent care (see, Aleckson et al., 1986).

Laboratory Processing of Samples

Ideally, laboratories in which biological chromium analyses are done should be 'ultra-clean' facilities as described by Patterson and Settle (1976). Such facilities are appropriate for picogram level measurements of chromium in serum. Otherwise, a laboratory meeting

TABLE 4-1. SAMPLE COLLECTION AND PROCESSING FACTORS IN TOTAL CHROMIUM ANALYSIS

Step	Problem	Recommendation	References
General sample gathering	Potential for subtle routes of contamination	Use of well-trained, experienced trace analysis technicians in the field	Versieck et al. (1982) Behne, 1981
Sample drawing	Gross chromium contamination from needles and cutting tools	Use of plastic needle liner, cannulas, silanized needles, or non-steel blades	Versieck et al. (1979) Versieck et al. (1982) Veillon et al. (1984)
Sample collections	Chromium contamination of samples by element in containers especially contamination of serum and urine	Use of quartz tubes specially cleared and coated; use of tested chromium-free plastic ware	Fell et al. (1980) Halls and Fell (1983)
Laboratory processing	Requirement for 'ultratrace' or 'Class 100' facilities	Results problematic if specially qualified facilities are not used	Patterson and Settle (1976) Ottaway and Fell (1986)
Sample handling in laboratory	Reagent contamination and chromium loss	Chromium-free reagents or nonchemical degradation required	Versieck et al. (1979) Kumpulainen (1984) Veillon et al. (1982)

'Class 100' standards should be used. Finally, only highly trained, experienced laboratory personnel using chromium-free reagents and laboratory ware and using laboratory performances assessment procedures (see below) should be employed in such analyses. Reagents for chromium analysis are a chronic source of problems in the laboratory, requiring constant testing of chromium-free-certified reagents. Methods which minimize or avoid reagent use are desirable if they also meet traditional analytical performance criteria.

Serum chromium analysis is especially demanding since this medium usually has chromium levels lower than other biological matrices (Versieck et al., 1979). Dry ashing at elevated temperature is preferable if wet reagent decomposition produces chronically high and unacceptable blank values (Ottaway and Fell, 1986). Similarly, urinary chromium analysis by direct sampling can be used with appropriate safeguards (Kumpulainen et al., 1983; and Veillon et al., 1982).

Potential loss of chromium during sample degradation steps has plagued many methods and laboratories (Ottaway and Fell, 1986). Careful wet ashing of media with mixtures of nitric-perchloric-sulfuric acids can minimize chromium loss during mineralization. The charring step in conventional muffle furnace ashing is acceptable below 500°C while the charring step in the graphite furnace of atomic absorption spectrometry is acceptable below 1200°C.

4.1.2 Instrumentation Methods

One can divide instrumental methods for total chromium analysis into those involving atomic absorption/emission spectroscopy and those involving nonatomic spectroscopic techniques. The former principally includes atomic absorption spectrometry in the flameless (electrothermal atomization-atomic absorption spectrometry, ETA-AAS) mode and the newer method of inductively coupled plasmatomic emission spectrometry (ICP-AES). For this discussion, the latter will include X-ray fluorescence analysis with various modes of excitation and various configurations of neutron activation analysis.

Hundreds of chromium methodology studies have been described in the literature over the years, either as chromium-specific or multi-element measurements. The broad features of these various methods have been reviewed (e.g., Anderson, 1981; Kumpulainen et al., 1984). The vast majority of these have not been evaluated critically regarding their accuracy and

precision by present laboratory practice criteria. It is also the current view (e.g., Veillon, 1988; Ottaway and Fell, 1986) that limitations in methods before 1978 or so, particularly those using ETA-AAS, are not reliable, and values of concentrations are too high. Consequently, only the most recent representative techniques are presented in this update material.

Atomic Absorption/Emission Spectrometric Techniques

The various atomic spectroscopic methods now applied to total chromium measurements differ markedly in their instrumentation specifics, their sample preparation requirements and their specifications of sensitivity, complexity of operation, and overall cost.

ETA-AAS and Related Methods

Two major factors which affect the usefulness of ETA-AAS for total chromium measurements at the trace and ultra-trace levels concern the adequacy of chromium detection limits in all sampling cases and spectrochemical interferences from matrix backgrounds in various biological and environmental samples.

The reported chromium detection limits for ETA-AAS range considerably, with a lower end to the range for final analytical solutions of ca. 0.1-0.2 μg/L (e.g., Ottaway and Fell, 1986). This detection limit is often adequate for urinary analysis of exposure subjects and measurements in contaminated environmental samples but may not be adequate for serum chromium analyses in unexposed human populations. Even this detection limit, 0.1-0.2 μg/L, demands very careful attention to analytical parameters using ETA-AAS, and may require use of a platform (Slavin et al., 1983) and pyrolytic graphite furnace tubes (Veillon et al., 1984).

The issue of background matrix interference in most commercially available ETA-AAS systems, especially those older systems using deuterium arc background correction, can be a serious one when dealing with very low chromium concentrations. Even at charring temperatures adequate to decompose organic matter, various inorganic salts will often remain to produce a background signal at the atomization step (e.g., Guthrie et al., 1978). It appears that background interference problems in the general flameless AAS analytical approach to human biological monitoring have contributed to earlier levels which are now known to be too high by at least 10-fold (Veillon, 1988).

The basic problem is the use of deuterium arc correction devices for controlling background interferences for analysis of an element such as chromium where the principal atomic absorption line is compensated poorly, i.e., 357.9 nm. The deuterium arc method generally is applicable for element lines at or below ca. 300 nm. To offset this problem, steps can be taken which in some cases further reduce the overall sensitivity. Use of a tungsten-halogen correction lamp will provide much better results, while adjusting balance in signals of older units to suppress background will enhance background noise.

Owing to the above interference problems, it is frequently not possible to do direct analyses of chromium in serum, urine, and other fluids. Although such forms of measurement are claimed periodically (see, e.g., Ping et al., 1983), most trace analysis laboratories prefer use of sample pretreatment steps. The trade-off is the level of reagent blank in chemical treatment steps versus interference problems from spectrochemical interferences.

As an alternative, O'Haver (1984) has attempted to enhance AAS sensitivity using a continuum, rather than a typical narrow-line, source via an Echelle spectrometer and a 300 W Xenon short-arc excitation continuum source. Detection limits appear to approximate that of narrow-line sources, and there is the added advantage of doing simultaneous analysis of other elements (Lewis et al., 1985).

Overall, AAS in the flameless mode is more appropriate for those samples where levels are >1 μg/L in final solution than for those below this reference value, as often encountered in human sera and natural waters. In the latter case, a high level of expertise and proficiency of the analytical staff are required.

ICP-ES Methods

This newer method, based on plasma excitation of the element atom and measurement of the resulting emission line(s), has made various inroads into the place of ETA-AAS methods for environmental sample analysis. Applications to biological monitoring of human populations have been more limited, due to relative sensitivities in methods. For occupational screening of urine samples, ICP-ES is probably adequate since its detection limit is 1-5 μg/L. This limit is one order of magnitude less sensitive than needed for clinical background

samples. One inherent difficulty is spectral interference which requires dilution of fluids with deionized water. This markedly reduces the level of sensitivity.

4.2 BIOLOGICAL/ENVIRONMENTAL APPLICATIONS OF ATOMIC SPECTROSCOPIC TECHNIQUES

Table 4-2 presents illustrative applications of atomic spectroscopic methods to biological and environmental media. ETA-AAS analyses of human biological fluids by both direct analysis and via sample pretreatment have been the basis of a large number of methodology reports in the literature. The approaches of Dube (1988) and Saryan and Reedy (1988) are typical of approaches using no matrix degradation. The report of Veillon (1988) shows a good detection limit for serum chromium using degradation of organic matrix. Levels of chromium in urine and serum are often measurable with ETA-AAS methods, especially in cases of chromium poisoning.

4.2.1 Neutron Activation and X-Ray Fluorescence Methods

These two types of instrumental analysis differ considerably from those described above. First, they have multi-element capability and, second, they tend to be more complex and expensive in their basic equipment packages. This is especially so for the different types of neutron activation analysis and those forms of X-ray analysis involving sample irradiation with high-energy beams, such as the proton beam (PIXE) and the synchrotron, linear polarized beam.

4.2.2 Neutron Activation Analysis of Chromium in Various Media

In traditional neutron activation analysis ashed larger samples or unmodified small quantities are irradiated in a neutron flux; the samples are set aside to permit decay of radiation; and the generated radioisotope quantitated radiometrically after chemical separations. Nuclear reactor irradiation of small amounts of processed biological material, using neutron fluxes of 1010-1015 neutrons/cm^2/sec. and bombardment times of up to 12 days and subsequent radiochemical measurement have been reported for chromium in

TABLE 4-2. BIOLOGICAL AND ENVIRONMENTAL CHROMIUM ANALYSES USING ATOMIC SPECTRAL ANALYSES

Medium/Method	Details	Results	References
Biological			
Human urine: ETA-AAS with Zeeman background correction	Direct analysis-chromium-spiked urine pool	Detection limit- ca. 0.1 μg/L; Precision: 10%	Dube (1988)
Human serum, plasma and urine: ETA-AAS with D_2 correction	Dilution with deionized water and matrix matching	Quite adequate for this clinical case of acute chromic acid ingestion	Saryan and Reedy (1988)
Normal serum chromium levels: ETA-AAS with D_2 correction	Partial chemical decomposition using HCl and $MgNO_3$	Detection limit=0.03 μg/L adequate for normal chromium levels in blood	Veillon (1988)
Mammalian soft tissue, bovine liver: ETA-AAS with in-situ treatment and D_2 correction	Direct degradation using modified C rod atomizer	Use of standard reference medium, SRM 1577, shows acceptable accuracy	Steiner et al. (1987)
Environmental			
Various foodstuffs: spinach and calf liver. Flame AAS with Co lamp background correction	Wet digestion with HNO_3, oxidation and extraction with MIBK	Detection limit=3.8 ng/ml analyte. Accuracy adequate using NBS SRM 1573 materials	Farre et al. (1986)
Chromium and other elements in airborne particulate: ICP-ES with background correction and 1 ml/min aspiration rate	Multiacid digestion ($HF+HNO_3$) of particulate on moderate volume air samplers ($HClO_4$ mixture)	Accuracy adequate using SRM 1648 particulate standard; detection limit: 2 ng/ml of acid analyte	Xiaoqun et al. (1987)

serum (Versieck and Cornelis, 1980), urine (Cornelis et al., 1975), lung tissue (Landsberger and Simsons, 1987), and bone (Grynpas et al., 1987).

The neutron activation approach is relatively tedious in that it requires sample-decay time sufficient to allow sample handling for subsequent isolation and/or analysis of the chromium species. The 51Cr isotope has a half-life of 27.8 days and a photopeak of 320 keV. The radiochemical separation technique is exacting. However, the direct, thermal/epithermal energy band analysis used in instrumental neutron activation analysis (INAA), which uses machine calculation of gamma-ray net peak areas and translation to chromium concentration, employs more technical equipment. Given the special requirements of this methodology in terms of equipment and operator expertise, its principal use is providing reference analysis data rather than routine measurements.

X-ray fluorescence analysis in its various configurations can bridge the gap between routine laboratory needs for chromium measurements and the specialized research or reference facility. The ordinary form of X-ray fluorescence analysis, be it energy-dispersive or wave-length dispersive as to detection configuration, is usually appropriate for analysis of larger quantities of sample having chromium levels in the parts-per-million (ppm) range and above. There are, however, newer variations on the conventional X-ray fluorometric approach which have somewhat more flexibility for chromium measurement in environmental and biological samples, especially in regard to sensitivity.

In PIXE X-ray spectrometry, samples under a high vacuum are irradiated with a proton beam of 2 to 3 MeV energy via a tandem Van de Graaff accelerator. Detection is by a low-energy photon detector fabricated with either a germanium or a lithium-doped silicon detector core (Tanaka et al., 1987; Maenhaut et al., 1987) and coupled with a pulse height analyzer interfaced with a microcomputer. Sample powders or ash can be used. In the PIXE approach, analysis error is introduced by overlap from large neighboring peaks. Using a variety of National Bureau of Standards (NBS) standard reference materials (SRMs), PIXE was shown by Maenhaut et al. (1987) to yield results which were within 5% of the reference values.

In the variant of total reflection X-ray analysis (TR-XRF) which is a form of energy-dispersive X-ray spectroscopy, ash and film samples are analyzed readily. The instrumentation configuration to achieve this includes an X-ray generator, a fine focus tube

and a multiple-reflection module. The high-energy portion of the primary X-ray beam is intercepted by the multiple-reflection module and only energy <20 keV is passed to impinge upon the optically flat support. When analyzing chromium at low levels, Compton and Rayleigh background scatter must be reduced to enhance detection power (von Bohlen et al., 1987). Detection limits are ppm or less, and the technique is as accurate as AAS and ICP-ES methods (e.g., Gerwinski et al., 1987).

A particularly sensitive variation of X-ray analysis uses the synchrotron beam which irradiates the sample. An intense, linearly polarized beam provides an irradiation fluorescence and permits use of tunable, wavelength-dispersive monochromators, which are inherently more sensitive than the conventional energy-dispersive system. The linear polarization feature also reduces the problem of background scatter. Overall, sensitivity is greater. However, the relative restriction of this irradiation source to physics research facilities limits its usefulness to conventional laboratories.

Illustrative applications of neutron activation analysis and X-ray fluorescence to biological and environmental chromium analysis are given in Table 4-3. Landsberger and Simsons (1987) examined samples of lung autopsy tissue using Canadian Medical Research Council reference material (hepatopancreas) to validate accuracy. The mean level of lung chromium was 8.6 ppm, dry weight. The mean value for the NAA analysis of reference material was 2.4 ppm vs. the reference value of 2.5 ppm. Bone ash representing cancellous bone from human femoral heads (N=23, 300 mg) was analyzed by instrumental neutron activation analysis with a detection limit of <5 ppm and a range of <5 to <10 ppm was found (Grynpas et al., 1987). Using PIXE with a 2.4 MeV proton beam and eight different NBS standard reference materials processed in two forms (form A was a powder, form B was an acid digest dried film), it was found that single-target multiple run precision was 2 to 5% for chromium and other heavy elements, and overall accuracy was within 5% of reference levels (Maenhaut et al., 1987). Total reflection X-ray spectrometry was used to analyze content of chromium and other elements in municipal waste incinerator ash in the form of either digests or leachates (Gerwinski et al., 1987). The levels of chromium in both forms were validated by comparisons with AAS and with ICP-ES. Synchrotron irradiation X-ray analysis was tested for its use in measurement of chromium and other metals in biological materials using SRM milk powder as a reference material (Giauque et al., 1987). The X-ray

TABLE 4-3. RECENT NEUTRON-ACTIVATION AND X-RAY FLUORESCENCE ANALYSIS FOR CHROMIUM MEASUREMENTS

Method	Samples	Results	Reference
Thermal/Epithermal Neutron Activation Analysis (NAA)	Lung tissue from autopsy (N = 5)	Mean chromium = 8.6 μg/g (dry weight). Excellent (2.4 ppm) accord with reference pancreas tissue (2.5 ppm)	Landsberger and Simsons (1987)
Instrumental NAA (INAA)	Cancellous bone from human femoral heads (N = 23); as bone ash, 300 mg	Detection limit ≤5 ppm Range in samples ≤5 ppm-≤10	Grynpas et al. (1987)
PIXE: 2.4 MeV proton beam	Various standard reference matrices (N = 8)	Chromium levels were well matched with SRM values; 5% error; replicability of single analysis. Overall accuracy, 5%	Maenhaut et al. (1987)
Total Reflection X-ray analysis	Municipal waste incinerator ash using digests and leachates	Compared with AAS-ICP gives excellent comparability measured as correlations	Gerwinski et al. (1987)
Synchrotron X-ray analysis	Validation of method through standard reference materials, milk powder	Chromium <0.6 ppm vs. 0.003 NBS value, 500 mg	Giauque et al. (1987)

method showed chromium as <0.6 ppm vs. an NBS reference value of 0.003 when samples of 500 mg were used.

4.2.3 Quality Assurance/Quality Control (QA/QC) Protocols

The elements of an idealized QA/QC framework include close adherence to the use of internal and external control materials and other proficiency standards (Aitio and Jarvisalo, 1984; Schelenz et al., 1989). A number of standard reference materials which have been validated for chromium and should be an integral part of any laboratory's QA/QC program, are summarized in Table 4-4.

Chromium levels in a variety of standard reference materials are known. These include samples from such sources as the National Institute of Science and Technology (formerly NBS) SRM program and the many samples of the International Atomic Energy Agency (IAEA) program. The IAEA program has a total of 11 samples for chromium validation. Of these, six are clinical/biological in nature, and five are for environmental assessment.

Overall, the data in Table 4-4 indicate that when many laboratories using many approaches are evaluated for chromium analysis, the variability is large as one gets down to the low levels in biological media. This is seen when one examines results of the IAEA H-9 human diet testing, using seven laboratories and three of the most commonly used methods.

4.3 CHEMICAL SPECIATION ANALYSIS OF CHROMIUM IN BIOLOGICAL AND ENVIRONMENTAL MEDIA

The various chromium valence species and other chemical forms show marked differences in biological activity. Hexavalent chromium [Cr(VI)] is assumed to be more toxic than trivalent chromium [Cr(III)] owing to the relatively potent carcinogenic character of various salts in the lung.

Whatever the value of chemical speciation analyses, they are also fraught with analytical hazards. This is because of the valence lability of Cr(VI) various reactions to include: environmental transformations of Cr(VI) to Cr(III) or the reverse once emitted to various environmental compartments; the *in vivo* conversion to the rather more innocuous trivalent state after filtration to urine or in other media, e.g., lung fluids and blood; and artifactual

TABLE 4-4. RECENT USES OF STANDARD REFERENCE MATERIALS IN CHROMIUM ANALYSIS

Reference Material/Source	Methodology Used	Results	References
Chromium in mixed human diet, IAEA No. H-9	7 laboratories using ETA-AAS, ICP-ES, and NAA	There is considerable error, i.e., wide confidence intervals, in all methods for chromium. Each method had specific problems	Schelenz et al. (1989)
Chromium in NIST (NBS) milk powder	Neutron activation with chemical separation	Certified value- 2.6 ng/g This method- 2.6 ng/g	Greenberg et al. (1988)
Chromium + other metals in lyophilized and natural urine samples; Institute of Occupational Health, Helsinki, Finland	12 laboratories: AAS in various analytical configurations; background and 2 spiked samples to each laboratory	Chromium recovered, 96.5 and 94.3%, in both urine forms, r=0.978 C.V. = 8-24% for 385 nmol/L spike	Valkonen et al. (1987)
Chromium + other metals in soil SO-1 reference material: Academy of Mining and Metallurgy, Kracow, Poland	30 laboratories using AAS (41%), INAA (36%), X-ray spectrometry (8.3%), emission spectrometry (5.7%), and miscellaneous (9%)	Range 23-58 ppm, Rel. S.E. = 11.6% C.I.=29.4-47.5 ppm for AAS, INA, ES	Holynska et al. (1988)

transformation between valencies during analysis at lower pHs (reduction) or the reverse in alkaline media conditions.

Harzdorf (1987) has classified two types of chromium speciation techniques presently in use: those which measure one or more forms directly, e.g., spectrophotometry and electrochemical techniques, e.g., polarography. In these cases, hexavalent chromium has discrete spectral and electrochemical characteristics.

The majority of the methods are those where specificity is based on separation of forms, e.g., Cr(VI) from Cr(III) inorganic anions and followed by some metal-specific but not form-specific detection, e.g., ETA-AAS. With form-specific detection speciation efficiency is a matter of separation power for the valency forms. Chelation-extraction separations, using such common agents as 1,5-diphenylcarbazide or its derivatives, are based on complexation between the chelant, the hexavalent form, and measurement of chromium through ETA-AAS. Chromatographic separations are also known, using ion chromatography in its various configurations. Illustrative recent applications of the above types of analysis for biological and environmental analyses are presented in Table 4-5.

Chromium speciation of the hexavalent and trivalent states in serum has been reported by Urasa and Nam (1989) using NBS reference serum for comparison. Separation was by cation/anion dual chromatography with a plasma emission detector for specific quantitation. The total chromium value, which is what was actually certified and not various forms therein, accorded closely to the reference material, while speciation gave a roughly 50:50 distribution of Cr(VI) vs. Cr(III) with a sum close to the reference total.

Other biological media have been subjected to attempted speciation via chromatographic approaches. Minoia and Cavalleri (1988) used anion exchange chromatographic separation with an AAS detector to examine urine for forms of chromium present. No Cr(VI) was detected which would be expected based on earlier studies. Similarly, Suzuki (1987) combined anion-exchange high-performance liquid chromatography with AAS detection to provide a sensitive system (2-5 ng detection limit) for examining reducing activity *in vitro* of Cr(VI) in various experimental animal media. In rat plasma, hemolysate and liver supernatant, various rates of Cr(VI) reduction were measurable. These data further support the notion that chromium valency is quite labile in mammals. Direct measurement of Cr(VI) in serum was done using the method of polarography as described by Harzdorf (1987) for the

TABLE 4-5. RECENT CHEMICAL SPECIATION METHODS FOR CHROMIUM IN VARIOUS MEDIA

Speciation Level/Medium	Methods	Results	References
Cr(VI) and Cr(III) in NBS SRM serum (SRM 909) and natural water (SRM 1643(b)).	Cation/anion tandem ion chromatography with D.C. Plasma emission Cr detector	Serum: total chromium matches SRM very well. Cr(III)=.06 μg/ml Cr(VI)=.05 μg/ml	Urasa and Nam (1989)
Attempted Cr(VI) levels in urine of workers exposed to Cr(III) or Cr(VI)	Liquid anion exchange chromatography + ETA-AAS detection	No urinary Cr(VI) seen in any case, i.e., rapid reduction *in vivo*	Minoia and Cavalleri (1988)
Cr(VI) and Cr(III) complexes from rat plasma, hemolysate and liver supernatant after adding both forms *in-vitro*	Anion-exchange high performance liquid chromatography with AAS detection	Reduction rate of Cr(VI) readily followed in these systems. Detection = 2-5 ng Cr	Suzuki (1987)
Cr(VI) in serum, water sediments and waste water	Electrochemical: polarography at pH 9-12	Cr(VI) can be measured in water but rapid, partial reduction occurs in sediments, some reduction in serum	Harzdorf (1987)
Cr(III) and Cr(VI) in drinking water supplies	Chelation-extraction at adjusted pH using APDC-MIBK and ETA-AAS as detector	Cr(VI) is measured directly and Cr(III) is obtained by difference	Subramanian (1988)
Cr(VI) in welding fumes, using standard samples for different welding systems (Danish Welding Institute)	Anion exchange chromatography with ETA-AAS	Using reference samples, this method gives an overestimate of ca. 30%	Brescianini et al. (1988)

pH range of 9-12. The detection limit for the method is 20-50 parts-per-billion. This approach was also applied successfully to waste water, sediments and river waters. The results show also that serum will reduce Cr(VI) when it is added as the simple anion.

Environmental applications of form-specific methodology are known also. Besides those mentioned already, Subramanian (1987) applied chelation-extraction to measurement of Cr(VI) in tap water supplies, using ammonium pyrrolidine carbodithioate in methylisobutyl ketone as the extraction system. The level of Cr(VI) in welding fumes is of particular concern from a worker health perspective, and a number of reports have described analyses to measure such a form. In the illustrative approach of Brescianini et al. (1988), anion-exchange chromatography in tandem with ETA-AAS as a metal detector was used, and the method was examined using reference fume material from the Danish Welding Institute.

In general, the value of speciation may lie more in environmental assessment than examining *in vivo* behavior of specific forms taken into the body. It is known for example, that one cannot easily examine chromium workers for form-variable chromium exposures, since chromium from all sources is found in urine as the trivalent form (Minoia and Cavalleri, 1988). Enough of a reduction potential exists that one cannot relate form-variable chromium in serum to exposure or biotransformation rates (Harzdorf, 1987). Thus, it would not be useful to speciate chromium in human tissues since reports show significant reduction of Cr(VI) in tissue and cell preparations (Suzuki, 1987).

The application of methods for environmental speciation, however, would be governed by the relative red-ox power of the medium in which the chromium is found. Chromium in complex waste mixtures where oxidizable material is mixed with Cr(VI) along with acid wastes would be apt to eventually convert to reduced element. Similarly, alkaline wastes co-occurring with Cr(III) and strong oxidants would proceed toward oxidation over extended time. "Chromium-clean" systems may be more amenable to speciation analysis, taking into account the long timeframes in which chromium is apt to be present in such media.

4.4 SUMMARY AND OVERVIEW

Total chromium analysis in biological media is feasible if full account of the many analytical hazards are taken. For chromium in serum of the general population, specialized

laboratories using highly-stringent techniques are probably required. Urinary chromium and chromium in biological samples other than sera/plasma are higher in concentration and are more forgiving of laboratory limitations.

With these caveats in mind, it appears that flameless AAS is probably the best method for total chromium analysis in samples where extremely high sensitivity is not needed. Other methods have been noted, although they become increasingly more complex and less routine in application as one proceeds through the list. ICP-ES is generally less sensitive than ETA-AAS, but it is a multi-element measure; and this may be of considerable value in some cases. Neutron activation analysis with or without laborious chemical separation steps is mainly a reference procedure as are some of the X-ray fluorescence approaches using high-energy irradiation beams to activate chromium.

Chromium is still a trace and ultra-trace element in most media, and its analysis requires use of good laboratory practices and a stringent QA/QC protocol. Such a protocol should partake of the variety of standard reference materials for biological and environmental method validations.

Chromium speciation techniques are feasible, but their value to biological assessments are circumscribed owing to valence state lability of Cr(VI) and Cr(III) in bioredox systems. Urinary chromium does not preserve original exposure forms, especially in the workplace.

Environmental applications may be more promising if one can first characterize the pH of the entire environmental medium and the presence of other redox-active species. Some environmental media may be simple enough to permit speciation e.g., population exposures through inhalation of airborne Cr(VI) particulate. However, valence stability of chromium on collection filters in high-volume air samplers or even personal samplers would have to be validated.

5. COMPOUND DISPOSITION AND KINETICS

Human beings are exposed to two main forms of chromium. Trivalent chromium, Cr(III), is a stable form which as an organic complex is present in some foodstuffs and is regarded as an essential factor in insulin-mediated glucose metabolism. Cr(III) also occurs in food bound to proteins. The body stores of chromium in the general population are derived from dietary intakes. Air exposure results in an accumulation in the lung with age (Schroeder et al., 1962; Kollmeier et al., 1985; Raithel et al., 1988; Pääkkö et al., 1989) due to the strong complex binding properties of Cr(III). Hexavalent chromium, Cr(VI) only exists in oxyanions as hydrochromate, chromate, or dichromate. Exposure in the working environment is via air and sometimes via skin. Small amounts also may be present in the ambient air. Chromate ions will pass easily through membranes utilizing the same pathways as sulfate ions.

The term Cr(VI) will be used in the following text, but it is the fate of the chromate ion, CrO_4^{-2}, which is being described. The reduction mechanism of Cr(VI) to Cr(III) must be understood before describing chromium metabolism. This topic was briefly discussed in the 1984 HAD, but extensive research since the completion of that document makes it possible to give more valid information. This information is needed for risk evaluation.

5.1 MECHANISMS FOR REDUCTION OF HEXAVALENT CHROMIUM COMPOUNDS

Section 3.3.1 mentions that in ambient air Cr(VI) will be reduced to Cr(III). Oxidation of Cr(III) to Cr(VI) is less likely to occur. The fate of chromium in soil was discussed in Section 3.3.2, Cr(VI) will be reduced to Cr(III) in acid soils, but the possibility of Cr(III) oxidation to Cr(VI) by oxidizing agents, e.g., manganese oxide, was pointed out.

Hexavalent chromium compounds may be inhaled and absorbed in workplace environments and in ambient air near point sources. These compounds will be subjected to several reducing environments in the respiratory tract. The fate of hexavalent chromium has been discussed by Petrilli et al. (1986a). Some reduction to Cr(III) may occur in the lumen

of the terminal airways by contact with epithelial lining fluid. In the alveolar region, human and rat macrophages have the capacity to engulf Cr(VI) compounds in particle form and also the capacity to reduce Cr(VI) to Cr(III), which eventually will be transported away by mucociliary transport (Petrilli et al., 1986b). Some chromium will be transported by macrophages via the lymph to lymph nodes. It is known that hilar lymph nodes of humans contain high concentrations of chromium (Bartsch et al., 1982). The reducing capacity of alveolar macrophages is even higher than that of liver cells (Petrilli et al., 1986b). Cells from bronchial or lung tissue of rats and humans seem to have some capacity to reduce Cr(VI), but much less than that in the liver (Petrilli et al., 1985). This intracellular reduction is enzyme-catalyzed similar to what has been seen in liver cells. Further evidence for reducing mechanisms in the lung has been provided by Suzuki (1988) and Suzuki and Fukuda (1989), who studied the reducing capacity of cell-free lavage fluids from rat lungs and postulated ascorbic acid, which is present in the surfactant layer of the alveoli to be one important reducing agent. Suzuki (1988) estimated that the ascorbic acid present could reduce about 8 μg Cr(VI) per gram of lung tissue, which was higher than the capacity of liver cells; no comparison was made with lung macrophages. Most Cr(VI) is reduced to Cr(III) in the respiratory system before Cr(VI) can penetrate into lung tissue cells. If the reducing capacity is overwhelmed, as may occur in occupational exposure, additional soluble compounds of Cr(VI) may be absorbed by these cells and result in systemic absorption.

The ingestion of Cr(VI) for instance as small amounts in drinking water, raises the question of whether any will be absorbed in that form since the saliva and gastric juice of humans seems to have a high capacity to reduce Cr(VI) to Cr(III) (De Flora et al., 1987; De Flora et al., 1989). Donaldson and Barreras (1966) found that after a tracer dose of $Na_2{}^{51}CrO_4$ was administered p.o. to fasting humans about 2% of the dose was found in urine compared to 0.5% after a dose of Cr(III), thus, some chromate was absorbed. After intraduodenal infusion in four patients, more chromate was absorbed than after passage through the stomach. The acidity of gastric juice was an important factor since less Cr(VI) was reduced by achylic persons (Donaldson and Barreras, 1966). De Flora et al. (1987) observed that while the reduction of Cr(VI) was favored by the acidic gastric environment, the reaction appeared to depend on the presence of thermostable reducing agents in the gastric juice, the presence of which varied widely according to the intake of food.

Oral uptake of Cr(VI) compounds may occur in occupational exposures, but assuming normal dietary habits even relatively large amounts may be reduced efficiently. The efficiency of Cr(VI) reduction is one factor for consideration. The rate of Cr(VI) reduction, as well as the time Cr(VI) is available for absorption is another factor for consideration. De Flora et al. (1987) showed that the amount of reducing agent present in gastric juice cycled with the patterns of food ingetion (the highest levels correlating with 3 to 4 hours after meals. On the other hand, Cr(VI) reduction was less pronounced by a factor of ten in fasting individuals and at night irrespective of pH variations. De Flora et al. (1987) estimated that more than 10 mg of Cr(VI) could be reduced daily by human gastric juice although the kinetics of the system with this capacity were not discussed.

Cr(VI) absorbed into the blood will be reduced in the plasma by proteins. Ascorbic acid also may play a role (Korallus, 1986). These results were obtained by experimental studies on human serum. Ascorbic acid also is effective in the treatment of acute poisoning by Cr(VI) compounds (Korallus et al., 1984). One liter of plasma can reduce 2 mg of Cr(VI) to Cr(III) (Korallus, 1986). Experience from studies on exposed workers indicates that circulating Cr(VI) is filtered through the glomerulus and excreted. Slight renal tubular damage occurs at exposures resulting in low blood or plasma levels. The plasma reduction rate *in vivo* may not always be rapid enough for complete reduction. Further evidence for the beneficial effects of ascorbic acid is given by Ginter et al. (1989) who showed that ascorbic acid protected against toxicity from orally administered dichromate in guinea pigs (see also 6.7).

Cr(VI), which has not been reduced in the plasma, may pass into the red cells. Cr(VI) will be reduced to Cr(III) in the red blood cell by binding to hemoglobin and thiols, especially glutathione (Lewalter et al., 1985).

Table 5-1 shows the distribution of chromium in rats given intratracheal installations of 3.5 and 87 μg of radioactive Cr(VI) as sodium dichromate (Weber, 1983). A large part of the dose is retained in the lungs – the clearance being more rapid after the smaller dose. The concentrations are similar in serum, but in the erythrocytes a larger percentage of the dose is found after the higher dose. This indicates that the reduction in plasma was relatively more efficient after the lower dose.

TABLE 5-1. ACTIVITY (PERCENT OF DOSE) of ^{51}Cr IN RATS AFTER INTRATRACHEAL ADMINISTRATION OF Na_2CrO_4

	Day 3		Day 25	
Dose (μg Cr/kg)	3.5	87	3.5	87
Lung	31.7	32.7	10.0	18.5
Erythrocytes	0.34	0.94	0.20	0.49
Serum	0.30	0.31	0.0099	0.007
Liver	0.45	1.0	0.20	0.39
Kidney	1.7	2.2	0.80	0.97
Testis	0.090	0.094	0.066	0.065
Skin	1.4	1.8	0.64	0.76
G.I. tract	1.8	0.75	0.28	0.41
Residual Carcass	8.2	8.7	5.40	5.5
Whole body	45.9	48.5	17.40	27.1

(Source: Weber, 1983).

Reduction in plasma and red cells is, thus, a further step in detoxifying chromate ions. If any chromate ions are left in the circulation, they will either be excreted via the kidneys or taken up by other organs. Minoia et al. (1983) noted that reduction may occur in the urinary tract. Cr(VI) could not be found in urine from human beings exposed to chromates, however, Ginter et al. (1989) found that vitamin C protected against toxicity from orally administered dichromate in guinea pigs (see also 6.7).

Metabolism of Cr(VI) in liver cells has been extensively studied. A number of enzyme systems, thiols, and other reducing agents are involved (Wiegand et al., 1984a; Petrilli et al., 1985; De Flora et al., 1985; Connett and Wetterhahn, 1985; Mikalsen et al., 1989). Mitochondria, microsomes, and cytosol all have reduction capacity. According to De Flora et al. (1985), DT-diaphorase, a cytosolic enzyme, is mainly responsible for intracellular reduction. Cr(VI) is reduced in intact cells and microsomal and mitochondrial preparations, and a reactive intermediate Cr(V) is formed (Wetterhahn Jennette, 1982; Arslan et al., 1987; Rossi et al., 1988; Wetterhahn et al., 1989).

Thus, several different systems in the mammalian body are capable of reducing Cr(VI), but the capacity and kinetics of these systems, especially in the lung, are not well characterized.

5.2 ABSORPTION AND DISTRIBUTION

The absorption of Cr(III) from food seems to be dependent on dietary intake. Chromium absorption is inversely related to dietary intake. Anderson and Kozlovsky (1985) estimated that the absorption was about 2% when the dietary intake was 10 μg and about 0.5% at an intake of 40 μg, i.e., about 0.2 μg was absorbed daily. Urinary chromium excretion was not related to dietary chromium intake. However, daily chromium intake was less than 40 μg in this study. Similar results were obtained by Bunker et al. (1984) who reported net absorption of 0.6 μg when the intake was 24.5 μg. Increased chromium absorption with decreased intake is an efficient mechanism for ensuring relatively constant amounts of absorbed chromium.

With regard to absorption of inhaled chromium compounds, there are many factors to be taken into account. Cr(III) is slowly cleared from the lungs of guinea pigs, rabbits, and rats, and very little seems to be absorbed (Al-Shamma et al., 1979; Wiegand et al., 1984b; Edel and Sabbioni, 1985). The higher Cr(III) content in lung may come from the environment and remains undissolved (Hyodo et al., 1980). The increase in chromium levels with age in man indicates that retained Cr(III) may have a long half-time in the lungs (Schroeder et al., 1962).

The water solubility of Cr(VI) compounds is complex and ranges from compounds with high solubility, e.g., chromic acid (chromium trioxide), to those with low solubility, e.g., lead chromate. Animal experiments have shown that after exposure to soluble chromate (e.g., sodium chromate) via inhalation or intratracheal instillation, there is an uptake of Cr(VI) in blood (Langård et al., 1978; Bragt and van Dura, 1983; Wiegand et al., 1984b; Edel and Sabbioni, 1985), whereas lead chromate was poorly absorbed (Bragt and van Dura, 1983). Thus, biological monitoring of chromium in urine and blood of workers exposed to lead chromate may not be indicative of previous exposure. The chromium retained is likely to be in the reduced form. In all animal studies listed, the doses administered are much larger than the expected exposure to human beings from ambient air.

Absorbed chromium is mainly retained in the liver, spleen and kidneys. Chromium is absorbed from the alveoli, gastrointestinal tract, and skin as Cr(VI) (Hyodo et al., 1980). Muscle and fat retain only small amounts of chromium after exposure to Cr(VI). Such tissues do not have a reducing capacity. Five days after a single intravenous administration to rats of Cr(III) as chromium chloride, the highest concentrations of chromium were in the kidney, liver, spleen, and bone. The Cr(VI) distribution included the same four organs plus blood and intestine (Sayato et al., 1980). The plasma component of blood contains much more Cr(III) than the red cells (Edel and Sabbioni, 1985; Sayato et al., 1980). Cr(III) has a high binding activity for transferrin in plasma, and Cr(VI) is permeable into red cells where it binds to hemoglobin. In human beings without occupational exposure, the highest concentrations are in the lung. Among internal organs, the highest concentrations are in the liver, kidney, and pancreas (Hyodo et al., 1980). In a worker exposed to chromates who died 10 years after the last exposure, the chromium concentrations in the lung, liver, and kidney were several times higher than in controls (Hyodo et al., 1980).

Cr(III) is not absorbed after dermal exposure, but workers exposed to chromic acid and soluble chromates may absorb Cr(VI) via skin (Lindberg and Vesterberg, 1983a).

5.3 EXCRETION

Absorbed chromium, Cr(III) and Cr(VI), will be excreted mainly by the kidneys. Wiegand et al. (1984b) found that after intratracheal instillation to rats of about 0.1-0.5 mg of Cr(VI) as chromate or 0.1 mg of Cr(III) as the chloride about 16 and 8% of the dose, respectively had been excreted after 4 hours. Edel and Sabbioni (1985) gave groups of rats intratracheal installations of 0.01 mg Cr(VI) as chromate and 0.01 mg of Cr(III) as chloride, respectively. After seven days about 20 and 4% of the respective doses had been excreted via urine. Bryson and Goodall (1983) gave repeated weekly injections of 3.5 μg of Cr(III) and Cr(VI) to mice and found that after 14 weeks the body burden of chromium was nine times higher after Cr(III) treatment.

After intratracheal instillation chromium also will be found in feces, which is mainly due to mucociliary clearance from the respiratory tract to the gastrointestinal (G1) tract. In the rat study by Edel and Sabbioni (1985), 24 percent of Cr(VI) and 36 percent of Cr(III) administered by intratracheal instillation had been eliminated in the feces after seven days.

After injection of Cr(III) or Cr(VI) compounds, the excretion is via kidneys (Sayato et al., 1980). Sayato et al. (1980) showed that 25 days following injection of Cr(VI) and Cr(III) in rats, urinary excretion was twice fecal excretion — the urinary being 60% of the Cr(VI) dose and 50% of the Cr(III) dose. The excretion was more rapid in urine and feces for Cr(VI). Following oral administration the biological halflife for Cr(VI) was 22.2 days and for Cr(III) was 91.8 days.

There are also many studies on urinary excretion of chromium in workers exposed to different chromium compounds (Mutti et al., 1984; Randall and Gibson 1987; Angerer et al., 1987; Welinder et al., 1983; Sjögren et al., 1983; Kalliomäki et al., 1981; Aitio et al., 1984; Lindberg and Vesterberg, 1983a). Elevated concentrations of chromium (not speciated) in urine have been seen after exposure to Cr(III) sulfate (Aitio et al., 1984; Randall and Gibson, 1987) and after exposure to Cr(VI) compounds with excretion generally being much higher after exposure to soluble compounds (Angerer et al., 1987; Mutti et al., 1984; Lindberg and Vesterberg, 1983a). However, Aitio et al. (1984) concluded that in the tannery workers, absorption from the (G1) tract could account for most of the increase in urine excretion of chromium. Lindberg and Vesterberg (1983a) noted that among workers with obvious signs of dermal exposure to chromic acid, urine chromium was higher than expected from air levels.

Chromate can damage the renal tubules, and slight tubular dysfunction has been seen in workers exposed to Cr(VI) (Lindberg and Vesterberg, 1983b). This indicates that the glomerular filtrate can contain chromate, which will be taken up by tubular cells. Section 5.1 stated that Cr(VI) has not been found in urine from workers exposed to chromate, which provides evidence for reduction to Cr(III) in the renal tissue and in the urinary tract.

5.4 METABOLIC MODELS AND BIOLOGICAL HALF-TIME

No simple model exists for the kinetics of Cr(VI) in the lungs or after systemic absorption. Since the efficiency of the numerous reduction mechanisms may be dose-dependent, the fate of a large dose, such as in animal experiments or working environments, cannot be applied to exposures in the general population. Based on knowledge about the reduction kinetics of Cr(VI), it is likely that most Cr(VI) which can be inhaled from ambient air or ingested with drinking water will be reduced before systemic absorption can

occur. Even if there is absorption, further reductions to Cr(III) will occur in the blood and certain blood-receiving organs .

The lung is the critical organ for toxicity from inhaled chromium, and models may be developed in the future for the fate of Cr(VI) compounds in that organ. Type of compound, e.g., solubility, dose and pulmonary reduction systems are examples of factors that must be included in such models.

It is easier to predict the fate of the stable Cr(III). Onkelinx (1977) gave rats of different ages single intravenous injections of labelled $CrCl_3$, the dose was 0.76 μg of chromium per animal. The animals were followed up to four months. A three-compartment model was proposed. Plasma chromium had a short half-time during the first 6 to 8 hours, and then passed into a phase with a longer half-time. In bone, kidney, liver, and spleen, chromium was taken up and retained for longer periods. It also was noted that age played a role for chromium turnover, which fits with the finding that in human beings a decrease in chromium levels occur with age.

A human model was proposed by Lim et al. (1983), who gave six subjects a single intravenous injection of radioactive Cr(III) as the chloride and observed them for three months. The plasma pool was considered to be in equilibrium with three compartments having fast, medium, and slow turnovers. The slow compartment was mainly made up by chromium stored in the liver and had a half-time of 3 to 12 months.

The biological half-time of Cr(III) in rats after oral exposure was estimated to be 92 days (Sayato et al., 1980). In human beings a longer half-time of 192 days has been estimated (Sargent et al., 1979). As discussed by World Health Organization (1988), chromium turnover will be influenced by a number of factors, e.g., insulin and glucose.

6. HEALTH EFFECTS

It was shown in Chapter 5 that many factors are involved in the metabolism of hexavalent chromium [Cr(VI)] compounds. Type of compound and magnitude of dose are two important factors which must be taken into account when the health effects of chromium exposure are discussed. In Chapter 4, it was pointed out that it is difficult to determine accurately chromium species in air and other media, and it must be assumed that the exposure levels of Cr(VI) reported in some studies are not always accurate. In this chapter the main emphasis will be on the human health effects from exposure to Cr(VI), since it already has been well documented that low level exposure to trivalent chromium Cr(III) does not constitute a health hazard (U.S. Environmental Protection Agency, 1984a; World Health Organization, 1988). Since Cr(III) is regarded as an essential element, the essentiality also will be discussed. The carcinogenicity of Cr(VI) will be reexamined by the U.S. EPA's Carcinogen Assessment Group in a separate update document. Table 6-1 gives information on some studies which are not mentioned in the text or are only briefly discussed with regard to health effects, exposure, and kinetics. The basis for the risk assessment will be human studies. There also are a large number of toxicity studies on experimental animals. Some of these studies are summarized at the end of the chapter in Table 6-2.

6.1 THE ESSENTIALITY OF CHROMIUM

Experimental animal studies and clinical studies indicate that Cr(III) is needed for normal glucose metabolism and that chromium deficiency results in impaired glucose tolerance, which can be reversed by chromium administration (U.S. Environmental Protection Agency, 1984a). The basic mechanism is probably that chromium potentiates insulin in peripheral tissues; no data indicate that chromium plays a role in pancreatic insulin production (World Health Organization, 1988).

TABLE 6-1. SUMMARY OF INHALATION STUDIES ON HUMAN EXPOSURES TO CHROMIUM COMPOUNDS

Analytical Method	Occupation	Air Concentration of Chromium	Author's Findings/Statistical Significance	Reference
		A. STUDIES OF HUMAN EXPOSURE TO HEXAVALENT CHROMIUM OTHER THAN CHROMIC ACID		
NIOSH method 173. for Chromium NIOSH method 169 for Cr(VI)	Welders	Total chromium: 20 $\mu g/m^3$; Cr(VI): 6.0 $\mu g/m^3$	Significantly excessive prevalence of cardiovascular disease and a significantly increased prevalence of some respiratory symptoms (productive cough) among workers. Chromium was not identified as the only welding component contributing to the effects noted.	Johnson and Melius (1980)
ET/AAS ICP-AAS DPC for Cr(VI)	97 silicon steel rollers	Chromium = ND to 200 $\mu g/m^3$ Cr(VI) = 0.8 to 1.8 $\mu g/m^3$	Eye and nasal irritation were associated statistically with work in a dustier job; 4143% had mucosal symtpoms, 43% had recurrent cough, and 23% had chronic bronchitis.	Stephenson and Cherniak (1984)
ET/AAS Colorimetric	Catalyst plant workers at 2 plants	Total chromium: up to 37 $\mu g/m^3$ Cr(VI): 0.8 to 4.2 $\mu g/m^3$	69 to 72% of employees reported 1 or more symptoms [cough, nasal sores (20-35%), skin rashes (27-39%)], not clearly related to Cr(VI) exposure.	Zey and Lucas (1985)
ET/AAS	53 stainless steel welders (24 smokers)	Mean: 124 $\mu g/m^3$ Median: 103 $\mu g/m^3$	Air concentration of total chromium showed a linear relationship to post-shift urine concentration. Although a single urinary chromium measurement is not exact, such urine measurements can be used as a crude estimate of airborne exposure Tendency for smokers to have higher urine concentrations. No relationship between years in occupational welding and urine chromium concentrations, but results show that current and previous exposure contribute to urinary chromium.	Tamino et al. (1981)
ET/AAS	Manual metal arc stainless steel welders	Total chromium: 81 $\mu g/m^3$ Cr(VI): 55 $\mu g/m^3$	Welders had far higher levels of chromium in urine than individually matched controls both in morning and afternoon. Nephrotoxic effects were not investigated. There was no observed cytogenetic effects in blood lymphocytes, and no apparent cytological evidence for mutagenicity.	Littorin et al. (1983)

TABLE 6-1. (cont'd) SUMMARY OF INHALATION STUDIES ON HUMAN EXPOSURES TO CHROMIUM COMPOUNDS

Analytical Method	Occupation	Air Concentration of Chromium	Author's Findings/Statistical Significance	Reference
Spectrophotometric	Stainless steel welders	Not measured	Results show a slow and fast compartment for chromium elimination in urine. Slow = 14 days to infinity. Fast = 4-35 hr. Significant correlation ($p < 0.001$) between chromium in air and urinary chromium. However, the variation of urinary chromium to chromium air was considerable, especially at chromium air levels at or below 100 $\mu g/m^3$.	Welinder et al. (1983)
ET/AAS	Dichromate production workers	Cr(III): 48 to 1,710 $\mu g/m^3$ Cr(VI): 18 to 312 $\mu g/m^3$	Level of Cr(III) in the air failed to show any significant correlation to the urinary concentration of total chromium. Levels of chromium in urine of workers exposed to Cr(VI) are higher than those exposed to Cr(III) in spite of very large concentrations of Cr(III) in the air.	Minoia et al. (1983)
ET/AAS	MMA stainless steel welders	Total Chromium: 30 to 960 $\mu g/m^3$ Cr(VI): 11 to 340 $\mu g/m^3$	This study evaluated biomonitoring indices of chromium exposure; there were no health effects reported. The authors reported that total chromium and Cr(VI) in the atmosphere cause an increase of chromium in the blood. Use of chromium and Ni urinary analyses as indices of short-term exposure is not as dependable as previously assumed. The chromium and nickel concentrations in whole blood and plasma did not correlate with the measured exposure, but the daily mean increase in the chromium concentration reflected exposure to chromium and Cr(VI) very well. Retention rate of magnetic dust in lungs correlated well with the daily mean increase of chromium in blood. Good correlations found between the retention rate of magnetic dust and the personal air samples of chromium and Cr(VI).	Rahkonen et al. (1983)

TABLE 6-1. (cont'd) SUMMARY OF INHALATION STUDIES ON HUMAN EXPOSURES TO CHROMIUM COMPOUNDS

Analytical Method	Occupation	Air Concentration of Chromium	Author's Findings/Statistical Significance	Reference
ET/AAS NIOSH method 319 for Cr(VI)	Workers exposed to chromium	7-1,221 $\mu g/m^3$	This study reported the feasibility of urinary elimination of chromium as a biomonitor of chromium exposure; there were no health effects measured. Post-shift urinary chromium and its increase above pre-shift levels were closely related to the concentration of water-soluble Cr(VI) in the atmosphere. The authors concluded that both the post-shift urinary Cr(VI) and the difference between pre- and post-shift urinary Cr(VI) can be used to indicate exposure to Cr(VI): chromium (urine) = 10.62 + 0.384 Cr(VI)(air); ΔchromiumU = 3.88 + 0.167 Cr(VI)A. Similar relationships for Cr(III) were indicated but not confirmed. In workers exposed to water-insoluble chromates or to watersoluble chromic sulfate CrIII, urinary chromium was definitely higher than that observed in subjects not occupationally exposed to chromium compounds, but it cannot be recommended as a short-term exposure test for evaluation of the job-related hazard.	Mutti et al. (1984)
ET/AAS	Leather tannery workers	Workers: 4 to 29 $\mu g/m^3$ Controls: 1 to 3 $\mu g/m^3$	Urine chromium concentrations showed a workshift-related diurnal fluctuation. The diurnal fluctuation persisted during the vacation periods, indicating accumulation of chromium in the body. There was no correlation to creatinine and no indication of a relationship between Cr(III) in the atmosphere and chromium in urine. Chromium in bloodstream was transported exclusively by the plasma.	Aitio et al. (1984)
ET/ASS DPC for Cr(VI)	Welders	Total chromium: MMA: 3 locations (1) 30-160 $\mu g/m^3$ (2) 100-1,600 $\mu g/m^3$ (3) 45-300 $\mu g/m^3$ MIG: 60 $\mu g/m^3$ TIG: 10-55 $\mu g/m^3$	The study attempts to show the relationship between welding activities and chromium concentrations in the air actually breathed by workers. There were no measurements of health effects or human exposure. The welding fume concentration is proportional to the arc-time factor, defined as the percentage of shift time that the welding arc is operational. The correlation between the arc-time factor and the chromium concentration measured in the breathing zone appeared to be very poor. Of the MMA fumes, 50-90% of the chromium was Cr(VI). Less than 2% of the MIG and TIG fumes was Cr(VI).	van der Wal (1985)

TABLE 6-1. (cont'd) SUMMARY OF INHALATION STUDIES ON HUMAN EXPOSURES TO CHROMIUM COMPOUNDS

Analytical Method	Occupation	Air Concentration of Chromium	Author's Findings/Statistical Significance	Reference
NIOSH method 319 for Cr(VI) NIOSH method 173 to determine total chromium or chromium metal	Farm machinery painters/ coaters	Total chromium: 219 to 440 $\mu g/m^3$ Cr(VI): 109 to 445 $\mu g/m^3$	This study measured atmospheric Cr(VI) at several stations in the application of primer and paint to farm machinery. No biomonitoring or health effect data were collected. For the painter station, 85% of the measurements exceeded the NIOSH recommended exposure limit of 1 $\mu g/m^3$ of Cr(VI). Measurements of the 8 hr time weighted averages for total chromium were below the OSHA permissible exposure limit of 1 $\mu g/m^3$ for metal and insoluble chromium salts.	Bloom and Peguese (1985)
			B. STUDIES OF HUMAN EXPOSURE TO TRIVALENT CHROMIUM OR UNSPECIFIED CHROMIUM COMPOUNDS	
ET/AAS	Chromium pigment producers and ferrochrome workers	11 to 80 $\mu g/m^3$ (mean)	Cr(III) lignosulfonate dust was rapidly absorbed in the lungs, and a peak of urinary excretion was seen immediately after exposure. No appreciable accumulation occurred over three days. The addition of ethylenediaminetetraacetate or Cr(III) chloride to the urine of exposed persons greatly enhanced the capacity of chromium to traverse a dialysis membrane. It is concluded that Cr(III) lignosulfonate yields Cr(III) that acts pharmacokinetically like water-soluble Cr(VI).	Kiilunen et al. (1983)
NAA	Copper smelter workers	Not measured	A fourfold increase of chromium in autopsied lung tissue was found for smelter workers compared to controls. For retired workers, the concentration of chromium did not decline with time after exposure had ended, indicating a long biological half-life for chromium in lung tissue. Data on duration of exposure were inconclusive and could not demonstrate a clear relationship between chromium accumulation in lungs and duration of exposure. Because of the exposure to other metals, no definitive conclusion could be drawn concerning health effects.	Gerhardsson et al. (1984)

ND = Not detected
ET/AAS = Electrothermal Atomic Absorption Spectrometry
NIOSH = National Institute for Occupational Safety and Health
ICP = Inductively Coupled Plasma Emission Spectroscopy
NAA = Neutron Activation Analysis
DPC = Diphenyl Carbazide

TABLE 6-2. ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		A. STUDIES OF HEXAVALENT CHROMIUM		
		1. Inhalation Exposure		
Male Wistar rats of the TNO-W-74 strain	25, 50, 100, and 200 $\mu g/m^3$ air	Subacute: 28 days at 22 hr/day Subchronic: 90 days at 22 hr/day	Subacute exposure (28 days) to 25 and 50 $\mu g/m^3$ of chromium as sodium dichromate resulted in "activated" alveolar macrophages with stimulated phagocytic activities, and significantly elevated antibody responses to injected sheep red blood cells (BBC). Subchronic (90 days) low-level exposure had a more pronounced effect on activation of the alveolar macrophages, with increased phagocytic activities. However, at high Cr(VI) exposure level (200 $\mu g/m^3$), phagocytic function of the alveolar macrophages was inhibited. In rats exposed to this chromium aerosol concentration for 42 days, the lung clearance of inert iron oxide was reduced significantly. The humoral immune system was still stimulated at sub-chronic chromium aerosol concentrations of 100 $\mu g/m^3$, but significantly depressed at 200 $\mu g/m^3$ chromium. The authors concluded that low level exposure to Cr(VI) aerosols increases the respiratory defense and all measured immunological functions in an adaptive manner. At high Cr(VI) exposures, both alveolar macrophages and immunological functions are inhibited.	Glaser et al. (1985)
Mice	15 mg/kg body weight of potassium dichromate	Single intraperitoneal injection	Binding of Cr(VI) to liver cell fractions was studied to determine binding constituents. Liver concentrations of these constituents peaked at 2 hrs with most in the soluble, low molecular weight, fraction. This binding dropped at a faster rate than that in mitochondrial or microsomal fractions.	Suzuki and Wada (1982)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		A. STUDIES OF HEXAVALENT CHROMIUM (cont'd)		
Rat/male albino	0.05 g chromium/kg body weight	One injection daily for 20 days	Cr(VI) inhibits the activity of cellular enzymes in the kidney, especially alkaline phosphatase, acid phosphatase, glucose-6-phosphatase and lipase. These enzymes are active in cellular membranes and the membranes of cell organelles. The significance of the change in enzyme activity is controlled by the level of enzyme protein and lipids with consequent involvement of the cellular organelles.	Kumar and Rana (1984)
Mice/male	14 mg chromium/kg body weight	Single injection 5 min to 24 hr	Low molecular weight chromium binding substance (LMWCr) is distributed widely in the body, and quickly binds chromium in stable form at an organ site, especially in the liver. LMW Cr plays a large role in chromium detoxification. Other proteins involved with binding chromium are albumin and transferrin.	Yamamoto et al. (1984)
		B. STUDIES OF HEXAVALENT AND TRIVALENT CHROMIUM, OR GASEOUS MIXTURES		
		1. Inhalation Exposure		
Male rabbits	Cr(III): 600 $\mu g/m^3$ Cr(VI): 900 $\mu g/m^3$	4 to 6 weeks (5 days/wk 6 hr/ day)	Alveolar macrophages were collected at the end of the exposure period to measure the concentration, size, distribution, metal content, and function. Significantly more macrophages were obtained from the lungs of rabbits exposed to Cr(VI), but not from rabbits exposed to Cr(III), when compared with the controls. Macrophages from the Cr(III)-exposed rabbits contained round, dark inclusions rich in chromium. Macrophages from the Cr(VI) group showed less conspicuous morphological changes: enlarged lysosomes that contained short lamel-lae and electron-dense, patchy inclusions. Only Cr(III) produced functional changes of the macrophages, resulting in increased metabolic activity and reduced phagocytic activity.	Johansson et al. (1986a)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		B. (cont'd) STUDIES OF HEXAVALENT AND TRIVALENT CHROMIUM, OR GASEOUS MIXTURES		
		Inhalation Exposure (cont'd)		
Male rabbits	Cr(III): 300 $\mu g/m^3$ Cr(VI): 600 $\mu g/m^3$	4 to 6 weeks (5 days/wk 6 hr/ day)	Microscopic studies of lungs of half or more of the rabbits exposed to either Cr(VI) or Cr(III) showed intraalveolar or intrabronchiolar accumulations of macrophages. Both chromium forms, but especially Cr(III), produced enlarged lysosomes and accumulation of laminated structures in the macrophages (perhaps due to impaired catabolism of surfactant). After Cr(III) exposure, the lysosomes of alveolar macrophages also contained dark inclusions and the ability of cells to catabolize surfactants was thought to be imparied, although exposure time was not sufficient ot verify this conclusion. The histological changes observed after chromium were less marked than after $Ni^{2}+$ or $Cd^{2}+$ exposure.	Johansson et al. (1986b)
Rats/Sprague-Dawley	Cr(III): 8,000-10,000 $\mu g/m^3$ Cr(VI): 7,400-15,900 $\mu g/m^3$	2 or 6 hr	Eight animals in Cr(VI) group died on or before the third day after exposure of severe asthmatic symptoms. No animals exposed to Cr(III) died. Cr(VI) caused weight loss and increased lung weight, due primarily to increased dry weight. Clearance of Cr(VI) from lungs depended on size distribution of particles: smaller particles (2627% less than 1 μm) had two phases, a rapid (T½ = 31.5 h) followed by T½ of 737 h in slower phase. Larger Cr(VI) particles (1214% less than 1μm) had T½ of 151 and 175 h. Cr(III) clearance was single phase of about 164 h, no matter what size. Blood chromium was much higher in Cr(VI) group compared to Cr(III) indicating faster removal from lungs with primary organ accumulations occurring in kidney and spleen.	Suzuki et al. (1984)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		B. (cont'd) STUDIES OF HEXAVALENT AND TRIVALENT CHROMIUM, OR GASEOUS MIXTURES		
		Inhalation Exposure (cont'd)		
Male rabbits	600 $\mu g/m^3$ or 3,100 $\mu g/m^3$ Concentrations that penetrate were 500 $\mu g/m^3$ or 1,900 $\mu g/m^3$	4 weeks (5 days/wk 6 hr/day)	Subsequent to exposure, alveolar macrophages were collected and tested for activity, viability, morphology and X-ray micro-analysis of metal contents. The macrophages from rabbits exposed to the higher concentration of chromium phagocytized significantly more particles than the controls. Chromium was actively phagocytized by the alveolar macrophages *in vivo*. Of the rabbits receiving the highest concentrations, 70% of all cells contained chromium. For low levels of chromium exposure, 12% of the cells in macrophage fractions contained chromium. Alveolar tissue cells contained no chromium.	Johansson et al. (1980)
Rats/Sprague Dawley	0.1 or 10 μg Cr(III) or Cr(VI)	intratracheal instillation killed at 29 hrs	Cr(III) taken up as such by organs may be distributed inside the cells differently from that generated by reduction of Cr(VI). Study suggests that the low-molecular-weight (LMW) components should be involved in the passage of this element from the lung to the other tissues.	Edel and Sabbioni (1985)
Rabbits/ New Zealand White	1. Cr(III): 0.1-0.5 mg 2. Cr(VI): 0.1-0.5 mg	1 dose as intratracheal instillation	1. Cr(III): found exclusively in plasma with peak at 20 min; decline in plasma is not monoexponential; plasma protein binding. 2. Cr(VI): predominantly in erythrocytes with peak at 120 to 180 min in plasma, slow decline with RBC's constant. Authors conclude that Cr(III) does not enter RBC's and Cr(VI) crosses lung unreduced. Total amount of Cr(III) remaining in lungs at end of experiment: 85%, compared to 47% for Cr(VI). Urinary values were 8 and 15.5% respectively. With exception of liver and kidney for Cr(VI), little chromium enters other organs.	Wiegand et al. (1984b)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		B. (cont'd) STUDIES OF HEXAVALENT AND TRIVALENT CHROMIUM, OR GASEOUS MIXTURES		
		Inhalation Exposure (cont'd)		
Rats/Wistar	Estimated 1.3 mg/m^3 based on MMA/SS welding fumes of 43 mg/m^3 at 3.6% chromium of total chromium, 95% is Cr(VI)	Intermittent for 2 hr/day up to 5 days	Chromium retention rate in lungs: 1.9 $\mu g/h$. After 10 hrs exposure, average chromium concentration in lungs was 39 ppm, corresponding to toal lung chromium content of 19 μg. Blood chromium was elevated in dose-dependent fashion; retention rate was 0.47 $\mu g/h$ with estimated maximum concentration of 4.7 μg. Chromium retention rates in kidneys and liver were 0.10 $\mu g/h$ and 0.19 $\mu g/h$ respectively. Liver chromium did not increase linearly with exposure and maximum concentration was 1.9 μg. Kidneys and spleen did continue to accumulate chromium, with maximum concentrations of 1.1 and 0.06 μg, respectively. From retained chromium in lungs, authors estimate that the soluble fraction of Cr(VI) may theoretically be as high as 95% of the total chromium, but only 30% of the total inhaled chromium is soluble in tissue. The authors further postulate that water-soluble, hexavalent alkaline chromates may undergo chemical reactions into insoluble chromium compounds in respiratory tract.	Kalliomaki et al. (1982)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		B. (cont'd) STUDIES OF HEXAVALENT AND TRIVALENT CHROMIUM, OR GASEOUS MIXTURES		
		Inhalation Exposure (cont'd)		
Rats/male Sprague/Dawley	Cr(III): 80 mg $CrCl_3$/kg Cr(IV): 20 mg $Na_2Cr_2O_7$/kg	Single i.p.	The distribution of chromium on chromatin and cytoplasmic RNA-protein (RNP) fraction in liver and kidney subsequent to the intraperitoneal injection of either Cr(VI) or (III). Cr(III) entered liver and kidney tissues at a slower rate than Cr(VI). Cr(III) did penetrate liver and kidney cells and was slowly bound to both RNP and chromatin. Cr(VI) was located on both the chromatin and RNP fraction in liver and kidney. Chromium on kidney chromatin peaked at 12 hr, in liver at 4 hr. For Cr(VI), DNA-protein cross-links were observed in both kidney and liver, but the time course was not correlated with the chromium binding to chromatin. Cr(III) complexes with the chromatin did not cause detectable DNA-protein cross-links, DNA interstrand cross-links, or DNA strand breaks. From these findings, the authors conclude the Cr(VI) in the kidney produced DNA damage, but not all types of chromium: DNA complexes produced DNA lesions.	Cupo and Wetterhahn (1985)
Rats/male Wistar	0.1, 0.5, 1 mg as $K_2Cr_2O_7$	Single intravenous injection	Cr(VI) was quickly reduced to Cr(III). At 5 min, only 2% remained as Cr(VI). Total chromium excreted in bile was primarily Cr(III) and was only 13.5 ± 2 μg/2 hrs at the highest dose administered. It is possible that the tri-valent form, bound to a low molecular weight protein from plasma, can penetrate liver cells and enter the bile.	Cavalleri et al. (1985)
Mice/C57BL	5 mg/kg body weight	1, 4, 24 hrs	Embryonic and fetal uptake of Cr(VI) was about 10 times higher than that of Cr(III). The Cr(VI) may have been reduced to Cr(III) before accumulation in the tissue. Uptake was more rapid during late gestation than before day 14, which marks the beginning of skeletal development.	Danielsson et al. (1982)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		B. (cont'd) STUDIES OF HEXAVALENT AND TRIVALENT CHROMIUM, OR GASEOUS MIXTURES		
		Inhalation Exposure (cont'd)		
Rats/Wistar	2 mg chromium/kg as $CrCl_3$ i.p., 3 x/week	15, 30, 45 or 60 days	Histologic changes in liver and kidney, with exposure-dependent increase in severity. Proximal tubular necrosis and hepatic parenchymal cell destruction. Changes in serum GOT, GPT, creatinine. Increase in serum urea.	Laborda et al. (1986)
	2 mg chromium/kg as Na_2CrO_4, i.p. 3 x/week	15 and 30 days	Ascites due to intrahepatic cirrhosis, with increased mortality beginning at day 18. All animals died prior to 60 days. Proximal tubular necrosis beginning at day 15; similar to that seen with Cr(III). Transaminases decreased severely; serum creatinine and urea shared similar pattern to that of Cr(III).	
Mice/NZC/CxO	Cr(III): 3.25 μg/g	3, 7, 21 days for mice with one dose	A single injection of 18 μg/g body weight of either Cr(III) or Cr(VI) can be lethal within 10 days, although the acute, three day toxicity of Cr(VI) is much higher than Cr(III). There was more evidence of cumulative toxicity for Cr(III). The authors concluded that the maximum tolerated dose for experimental purposes is 3 μg/g body weight given every 14 days, for Cr(VI). A lower dose would be required for Cr(III) due to its cumulative effect.	Bryson and Goodall (1983)
	Cr(VI): 3.23 μg/g	1 to 4, 8, 10, 12, 14 weeks for mice receiving repeated injections		
Rats/male albino ITRC colony	4 mg/kg	1, 7, 14 injections	Both Cr(III) and Cr(VI) can reduce the activity of certain hepatic and renal enzymes. The hepatic enzymes affected were anileine hydroxylase and aminopyrine N-demethylase, which are oxidative enzymes of the microsomal electron transport chain (nonooxygenases). The glutathione-S-transferase system also was affected, which could reduce the organism's defense mechanisms against carcinogenesis, mutagenesis, teratogenesis, and tissue necrosis.	Srivastava et al. (1985)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		B. (cont'd) STUDIES OF HEXAVALENT AND TRIVALENT CHROMIUM, OR GASEOUS MIXTURES		
		Inhalation Exposure (cont'd)		
Guinea pigs/ Rockefeller strain	0.07 mg Cr(VI) 0.07 mg Cr(III)	5 injections estimated 1/week	Both Cr(III) and Cr(VI) can sensitize guinea pigs. This sensitivity is persistent and appears to have the same determinant, or mechanism of sensitization. Guinea pigs sensitized with either Cr(III) or Cr(VI) are capable of reacting *in vivo* and *in vitro* to challenges with both chromium salts. This double reactivity is retained also after repeated restimulations with only onr of these chromium compounds. From the failure to select lymphocytes directed specifically against a chromium determinant of a specific valence, it is concluded that by sensitization with chromium salts of different valences, a common determinant or closely related determinants are formed, presumably by Cr(III).	Siegenthaler et al. (1983)
Rat/male Wistar-JCL	5 mg/kg	1 injection	There is a marked difference in the elimination kinetics of trivalent and hexavalent chromium. For subcutaneous injections, 36% of the Cr(VI) was eliminated in urine within seven days, compared to 10% for Cr(III). Fourteen percent of the Cr(VI) was eliminated in feces during the first seven days, compared to 24% of the Cr(III). The biological half-life for Cr(III) could not be determined for individual organs. For Cr(VI), each organ had two or three phases to the biological half-life curve, ranging from a fast phase of 2.5 hrs to a slow phase of 500 hrs. The slow phase for all major slow phase organs ranged from 200 to 500 hrs.	Yamaguchi et al. (1983)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		B. (cont'd) STUDIES OF HEXAVALENT AND TRIVALENT CHROMIUM, OR GASEOUS MIXTURES		
		Inhalation Exposure (cont'd)		
Rats/Wistar	32 µg/kg 225 µg/kg 960 µg/kg	5 hr	The relative amounts of chromium in bile are much lower after administration of Cr(III) than after Cr(VI) salts. Chromium appears as Cr(III) in bile after injection of a Cr(VI) salt, when doses from 32 to 960 µg chromium/kg rat weight are used. The reduction of Cr(VI) to Cr(III) is dependent on the availability of hepatic glutathione. Healthy liver cells rapidly reduce Cr(VI) to Cr(III); glutathione depletion of the liver with cyclohexene oxide decreased chromium excretion in bile.	Norseth et al. (1982)
Rats/male albino Charles River COBS Strain	0.1 or 100 mg Cr/rat 280-300 g/rat	24 hr	The route and mechanism of Cr(VI) elimination seems to be through binding with a LMW protein in the liver and elimination with bile. Cr(III) and Cr(VI) converted to Cr(III) appeared to be localized in cell organelles. The authors found very little CR(III) excreted through the bile duct. The presence of Cr(III) in the gastrointestinal tract, subsequent to bile duct ligation, suggests another route of elimination, possibly by excretion through the intestinal mucosa.	Manzo et al. (1983)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
			C. STUDIES OF TRIVALENT CHROMIUM	
Rats/Sprague Dawley	13.3 $\mu g/m^3$ particle size <2mm	5 hr, by inhalation	Lungs: 25-50 $\mu g/g$ wet weight Low molecular weight protein bound: 3 to 10% of total. High molecular weight protein bound: 60 to 70% of total. Liver: 2-3 $\mu g/g$ wet weight Low molecular weight protein bound: 56 to 71% High molecular weight protein bound: 15 to 25% Total chromium and high molecular weight chromium in lung declined with time with more chromium in the LMW fraction, so the level was approximately the same as the LMW chromium in the liver. Biological T½ for lung chromium: 12.8 days Biological T½ for liver chromium: 1.2 days Authors suggest LMW chromium in lungs is in equilibrium with rest of body chromium and participates in movement to other organs.	Wada et al. (1983)
Rabbits/male	0.6 ± 0.4 or 2.3 ± 1.1 mg/m^3 (mean ± SD) Cr(III) as $Cr(NO_3)_3\ 9H_2O$ (MMAD: 1 μm)	17 to 21 wks 5 x/wk, 6hr/day	Lungs: grayish color on plural surface in high dose. No Δ in wt. of lungs. High dose: nodular accumulation of macrophages in terminal airspaces; by electron-microscopy: macrophages had degenerative changes, enlarged lysosomes with membranous structures, inclusions which had high concentration of chromium; no changes in phosphatidylcholine and 1,2 dipalmitoylphasphatidyl choline indicating reduced catabolism of surfactant by alveolar macrophages.	Johansson et al. (1987)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		C. STUDIES OF TRIVALENT CHROMIUM		
Mice	0.2 ml of 0.08-0.78 mg/ml extracted from leather gloves	1. 3 hrs, subcutaneous injection	1. Skin irritation observed due both to low pH and chromium sulfate.	Naruse et al. (1982)
		2. 12 days, skin painting on depilated area	2. Variable skin reaction but high chromium level showed most irritation.	
Guinea pigs	0.25-0.50 mg/ml of Cr(III) or Cr(VI)	Skin maximization test, 6 days +	Cr(VI) solution produced a reaction; Cr(III) did not. Authors conclude that in humans wearing chrome-tanned leather gloves, contact dermatitis results from skin irritation from gloves followed by adherence of sweated-based chromium sulfate solution at low pH.	Siegenthaler et al. (1983)
Mice/female-pregnant ICAR/JCL	1. 9.8 mg single dose 2. 19.5 mg single dose	One i.p. injection	1. By 24 hrs blood chromium in fetus higher than maternal blood. 2. $CrCl_3$ caused cells of neuroepithelium of fetal animals to be extensively pycnotic. This was most evident at 8 hr postinjection. May result in neural tube defects, even exencephaly.	Iijima et al. (1983)

TABLE 6-2. (cont'd) ANIMAL STUDIES ON CHROMIUM EFFECTS, DISPOSITION, AND PHARMACOKINETICS

Species/Strain	Type of Exposure	Duration of Exposure	Author's Findings/Statistical Significance	Reference
		D. STUDIES OF OTHER VALENCE STATE OF CHROMIUM		
		Inhalation Exposure		
Rats/Sprague-Dawley, male and female	1) unstabilized CrO_2 0.5 mg/m^3 2) stabilized $CrO_{2,3}$ 0.5 or 25 mg/m^3	6 hrs/day, 5 days/wk, for 2 yrs	No differences in weight gain or exposure-related mortality No differences in exposed animals wrt hematology, clincal biochemistry, or urninalysis parameters. Exposed's excreted more chromium than controls or exposed's lungs and mediastinal lymph nodes. Rats exposed to 25 mg/m^3 had high incidence of alveolar bronchiolarization with foamy macrophage response, cholesterol granulomata in 's rather than 's. Relevance to human exposure is considered remote.	Lee et al. (1988)

Lipid metabolism also may be affected by chromium deficiency, since low-density lipoprotein cholesterol (LDL-C), which is regarded as a risk factor in the development of cardiovascular disease, was lowered after chromium supplementation (World Health Organization, 1988).

Offenbacher and Pi-Sunyer (1988) recently have reviewed available data on chromium in human nutrition, and there are both positive and negative results with regard to the effect of chromium supplementation on glucose and lipids. The main problem is the lack of a good indicator for chromium levels in the body. Thus, it is difficult to select individuals with chromium deficiency. Of special interest for this document is whether excessive exposure to Cr(III) will have any influence on glucose and lipids. Such a study was performed by Randall and Gibson (1988), who determined insulin, total cholesterol, triglycerides, LDL-C, and HDL-C in serum from 72 tanner workers exposed to Cr(III). Compared to 52 controls with a similar age distribution there were no significant differences for any of these parameters. These workers had an average exposure time of about 11 years and elevated serum concentrations of chromium (Randall and Gibson, 1987).

Wang et al., 1989 studied subjects participating in a health screening program at a university. Thirty persons participated; 18 males and 12 females, ranging from 31 to 66, and all had serum cholesterol levels about 2000 mg/l. They were divided into three groups, 10 persons in each. No data are given on the age and sex distribution within these groups. One group was given supplements of 50 μg Cr(III) as the chloride 5 days a week for 12 weeks; another group was given yeast tablets corresponding to 9 μg Cr(III); the third group served as controls. There were no changes in insulin levels, but slight reductions were noted in LDL-C and total cholesterol in both chromium-supplemented groups, compared to the controls. It is difficult to assess the validity of the authors' claim that there were significant changes in the supplemented groups. The supplemented intake of complex chromium from yeast was quite low.

These studies support the conclusions by Offenbacher and Pi-Sunyer (1988). In people with normal dietary habits and without metabolic diseases, the intake of chromium is probably adequate for maintaining tissue concentrations of chromium so that glucose and lipid metabolism are not impaired. Excess chromium will not cause any metabolic disturbances.

The active compound, originally called "glucose tolerance factor", has not yet been wholly characterized. Recently, Yamamoto et al. (1989) isolated a chromium-binding substance from livers of rabbits who had received dichromate injections. This compound enhanced glucose oxidation and lipogenesis in adipocytes, and removal of Cr(III) resulted in loss of activity. The molecular weight of the chromium-binding substance was 1500, and it was rich in aspartate and glutamate.

A daily intake of 50-200 μg Cr(III) has been suggested in the 1980 RDA. Several studies in the United States have shown that the actual daily intake is probably between 25 and 75 μg (Offenbacher and Pi-Sunyer, 1988). It cannot be excluded that chromium deficiency may exist in certain populations, especially among elderly people.

6.2 RESPIRATORY EFFECTS

It has been well documented that high exposure to certain hexavalent chromium compounds, especially chromic acid (chromium trioxide), has caused severe effects on the upper airways (U.S. Environmental Protection Agency, 1984a; World Health Organization, 1988). Cr(VI) compounds at lower levels may cause irritation in the airways and functional impairment of the lungs. Evaluation of the levels at which such effects may occur previously have not been possible, however.

During recent years several papers have appeared, which together with some earlier data, make it possible to get a better understanding of effects from low-level exposure to Cr(VI). Studies have been performed on workers exposed to chromic acid during chrome-plating and on welders, which may be exposed to Cr(VI) when welding stainless steel. Studies on welders have given valuable information on the kinetics of Cr(VI) in human beings, but it is difficult to use such groups for an evaluation of respiratory effects since welding fumes contain many other gases and particles which may affect the respiratory tract. It also may be difficult to determine accurately the amount of Cr(VI) in such complex mixtures. In contrast, there is very specific exposure to chromic acid mist near the plating baths.

Thirty-seven chromeplaters were studied by Cohen et al. (1974). Total chromium in air was determined by atomic absorption spectrophotometry (no details given) and Cr(VI) by the

Abell and Carlberg (1974) method. As mentioned in Chapter 4 this method may give too low values due to reduction of Cr(VI). Samples were collected in the breathing zone of the workers (sampling time not stated). The concentration of total chromium varied from not detectable to 49.3 $\mu g/m^3$ and that of Cr(VI) from not detectable to 9.1 $\mu g/m^3$.[1] The mean concentrations were 7.1 and 2.9 $\mu g/m^3$, respectively. The workers were given a questionnaire and an examination of the nasal system and skin. Pulmonary function was not studied. The control group was 15 workers in the same plant but without exposure to chromium. Whereas none of the controls had a history of nasal sores, 62 percent of the exposed group reported that symptom. Also, other symptoms indicating severe nasal irritation were much more common among the chromeplaters. Inspection of the nasal mucosa showed that 95% of the exposed workers had pathological changes, compared to 7% among the controls. Eleven percent showed septal perforations. The severity of the mucosal changes seemed to increase with exposure time, but it is noteworthy that among workers exposed less than one year 57% had relatively severe changes. No attempt was made to relate individual exposures to nasal changes; and since air levels were measured only once, they would give little information on previous exposure. Assuming that the determination of chromium was relatively accurate, this study indicated that exposure to relatively low concentrations of chromic acid had caused severe local changes in the nasal mucosa. The authors pointed out that additional exposure resulted from transfer by hand from contaminated surfaces; thus, poor personal hygiene was an important factor.

A similar study on 11 chromeplaters was reported by Lucas and Kramkowski (1975). The concentrations of [Cr(VI)] varied from <1 to 20 $\mu g/m^3$ with an average of 4 $\mu g/m^3$. Nasal changes, including perforations, were noted, and poor hygiene resulting in direct contact was considered as an important factor in this plant. The levels of Cr(VI) reported in these two studies are lower than those reported earlier to cause nasal lesions.

More recently, Lindberg and Hedenstierna (1983) studied 104 chromeplaters from 13 workplaces. Air measurements with personal samplers were performed on 84 chromeplating workers on 13 different days. For the remaining 20 subjects, exposure was assumed to be similar to that measured for a fellow worker doing identical work in the same

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[1] In another section of that report the lowest concentrations are reported to be 1.4 and 0.019 $\mu g/m^3$, respectively.

areas. To evaluate the variations in exposure on different days, measurements were performed with personal air samplers on 11 subjects at three factories during an entire work week. Air measurements were performed with stationary equipment at five chrome baths during a total of 19 days. Sampling was done with glass fiber filters that were leached in an alkaline buffer solution at pH 12. After buffering to pH 4, Zephiramin was added, and the Zephiramin-Cr(VI) complex was extracted with methyl isobutyl ketone and analyzed by atomic absorption (Fukamachi et al., 1975). The limit of detection was 0.2 μg Cr(VI) per filter, which corresponded to a Cr(VI) concentration of 0.2 $\mu g/m^3$ during an 8-hr sampling period.

Forty-three subjects were studied with regard to the upper airways. They were exposed to chromic acid only with no exposure to other irritants. Exposure times ranged between 0.2 to 23.6 years. Nineteen office workers constituted a control group. When exposure was 1-1.9 $\mu g/m^3$ of Cr(VI), 4 of 10 workers complained of diffuse nasal symptoms, whereas none of 9 workers exposed to less than 1 $\mu g/m^3$ had such symptoms. Among 24 workers with mean exposures of 2-20 $\mu g/m^3$ there were more pronounced symptoms like stuffy noses and nosebleeding. Inspection of the nasal mucosa revealed mucosal changes in 11 of 19 workers exposed to less than 2 $\mu g/m^3$, atrophy in four cases but no ulcerations. None of the control group showed atrophy. Among 24 workers with higher exposure, atrophy was more common and 11 had ulcerations or perforations of the nasal septum. Ulcerations appeared in two subjects after less than one year of exposure. The data also indicated that peak exposures are of significance. This study by Lindberg and Hedenstierna (1983) confirms that nasal irritation and mucosal changes occur at relatively low concentrations of chromic acid and that there is a threshold around an average exposure of 1 $\mu g/m^3$.

There are a few earlier studies on lung function in chromeplaters. Bovet et al. (1977) and Franchini et al. (1977) made spirometric studies, but air concentrations of Cr(VI) were not measured. They used urinary chromium as an exposure index. Bovet et al. (1977) found that a high urinary chromium (>15 $\mu g/g$ creatinine) was related to a decrease in spirometric values (e.g., VC, $FEV_{1.0}$). Franchini et al. (1977) reported that 12 out of 18 workers had a decrease in spirometric values. The urinary chromium averaged about 17 $\mu g/l$ in that group.

Lindberg and Hedenstierna (1983) studied respiratory symptoms and lung function in 104 chromeplaters; some details of that study have already been given. Forty-three workers

were exposed almost exclusively to chromic acid, and 61 also were exposed to some other irritating chemicals. As controls, a group of 119 auto mechanics was selected. That group had been examined earlier with the same methods and by the same personnel as in the present study. To study short-term effects of exposure, spirometry was performed on Monday morning and Thursday morning and afternoon. In six nonsmokers with average exposures above 2 μg Cr(VI)/m^3, there were significant decreases in FVC, $FEV_{1.0}$, and FEF_{25-75} from Monday morning to Thursday afternoon as well as from Thursday morning to Thursday afternoon. The morning values were slightly lower on Thursday than on Monday, but the difference was not statistically significant. In 10 workers with average exposures below 2 μg Cr(VI)/m^3, there were no changes in spirometric values from Monday morning to Thursday afternoon. In 48 smokers there was a small but significant decrease in FVC, but there was no significant change in $FEV_{1.0}$ or FEF_{25-75}.

To study long-term effects of exposure to chromic acid the Monday morning values for nonsmokers and smokers were compared to the corresponding values from the control group. No differences between the groups was demonstrated. An additional analysis was made by multiple linear regression taking height and age into account. Exposure time correlated well with age, but no significant difference between the exposed group and the control group was shown. This study indicates that reversible effects on lung function can occur when exposure is above 2 $\mu g/m^3$, but chronic effects do not seem to occur even after long-term exposure.

6.3 RENAL EFFECTS

Injection of chromates into experimental animals has been used to produce renal tubular damage. Early in this century several cases of renal damage after accidental ingestion or therapeutic applications of Cr(VI) compounds were reported (U.S. Environmental Protection Agency, 1984a).

Thus, renal effects have been searched for in exposed workers. Mutti et al. (1979) reported an increased excretion of beta-glucuronidase and protein in welders and chromeplaters with high urinary chromium (above 30 μg/g creatinine). This concentration may correspond to at least 10 μg Cr(VI)/m^3 in the air.

Littorin et al. (1984) studied a group of welders with an average chromium excretion of 6 μg/g creatinine (morning) and 11 μg/g creatinine (after work). They determined several indices of tubular toxicity but did not find any evidence of tubular dysfunction. The levels of Cr(VI) in air were not reported.

Lindberg and Vesterberg (1983b) examined the urinary excretion rate of proteins in 24 men currently working as chromeplaters, 27 former chromeplaters, and 37 referents who were divided by age for comparison with each of the chrome workgroups. Some of the men in the referent group were used in each comparison based strictly on their age distribution. The chromeplaters ranged in age from 20 to 70 years with a mean age of 36. Their chromium exposure by air was monitored by personal exposure monitors. The 8-hr mean value ranged between 2 and 20 $\mu g/m^3$ and averaged 6 $\mu g/m^3$. The exposure period ranged between 0.1 and 25 years with the mean of 5.3 years and median of four years. The former chromeplaters had worked in less modern plants between 1940 and 1968. The exact exposure level was not known but was presumably quite high since 7 of the 27 had permanent perforation of the nasal septum. The 37 men used as referents were selected from those working at the same company but not in the chromeplating facility. The men ranged in age from 18 to 80 years. Ten individuals over 60 were excluded to form the referent group for the current chromeplaters, and 10 of the youngest individuals were excluded, to form the referent group for the former chromeplaters. Thus, the age distributions of the four groups were similar though values obtained from 17 referents were used twice.

Urine collection was performed midway through the workweek. The urine was collected after the subjects had drunk a glass of water containing bicarbonate that promoted formation of alkaline urine; this was necessary to minimize the risk of degradation of $beta_2$-microglobulin. Protein analysis for urinary $beta_2$-microglobulin and albumin was performed by sensitive methods. The detection limit for $beta_2$-microglobulin was below 2 μg/l and <2 mg/l for albumin. In addition, all urines were analyzed for cadmium since concurrent cadmium exposure could result in elevated $beta_2$-microglobulin excretion. None were found to have cadmium exposure. Elevated $beta_2$-microglobulin (defined as >0.30 mg/l) was found in five current chromeplaters compared to one in their control group. There was a significant difference in the excretion of $beta_2$-microglobulin between current chromeplaters and their controls – the mean values being 230 and 150 μg/l, respectively. There was no difference

between former chromeplaters and their controls. Table 6-3 suggests a dose-response relationship between air levels and renal effects. The authors concluded this study demonstrates that renal toxicity may occur from chronic, low level chromium exposure. Theeffect may be reversible, however, since former chromeplaters did not differ from controls of similar age with regard to excretion.

Chromeplaters were also studied by Verschoor et al. (1988). Twenty-one chromeplaters and 38 welders exposed mainly to Cr(VI) were compared to 16 boilermakers exposed mainly to metallic chromium and 63 nonexposed workers. The average ages were 39, 41, 38 and 35 years, respectively. A large number of tests for both renal glomerular and tubular function were performed, e.g., creatinine and $beta_2$-microglobulin in serum, albumin, $beta_2$-microglobulin, retinol-binding protein (RBP) and some enzymes in urine. There were no abnormal findings with regard to tubular function or albumin excretion. Though the authors claimed a slight difference in glomerular function between the workers exposed to Cr(VI) and the other two groups, the data does not substantiate that the difference is of biological significance. The average excretion of chromium was 9, 3, 1 and 0.2 μg/g creatinine in the chromeplaters, welders, boilermakers, and nonexposed workers respectively which indicates that the chromeplaters had a relatively low exposure to Cr(VI). However, no data were given on air concentrations of chromium. This study offers further support of a threshold for renal effects of chromium.

Renal function was also studied by Franchini and Mutti (1988). The subjects were 43 workers with a mean age of 41 years, who were exposed to Cr(VI) in a plant producing chromates and dichromates. Exposure was high, since the median value for urinary chromium was 26 μg/g creatinine. Compared to a control group (n=30) all workers with urinary chromium below 15 μg/g RBP and albumin. In the group of workers with urinary chromium above 15 μg/g creatinine, the mean RBP excretion was about twice as high as in the control group. A dose-response relationship could not be established. Additional evidence for tubular damage was obtained by measuring renal antigens in the urine. In workers with high urinary chromium, a highly signficant increase in the excretion of these antigens were noted. A threshold for renal effects of chromium is supported.

Saner et al. (1984) reported a significantly lower excretion rate of urinary $beta_2$-microglobulin in tannery workers (n=18) and in a control group (n=16) used than in normal

TABLE 6-3. URINARY EXCRETION OF BETA$_2$-MICROGLOBULIN IN RELATION TO EXPOSURE LEVELS OF Cr(VI) AMONG PRESENT CHROMEPLATERS

Cr(VI) $\mu g/m^3$	Age N	Urine beta$_2$-m Mean	mg/l
11 - 20	5	39	0.23 - 130
4 - 8	13	37	0.04 - 0.44
2 - 3	6	29	0.06 - 0.18

Source: Lindberg and Vesterberg (1983b).

adults (n=12). However, essentially equivalent average urinary beta$_2$-microglobulin/ creatinine ratios were found for the tannery workers and the control group. The average urinary chromium concentration was 6.6 $\mu g/l$. There is no information on the level of exposure to Cr(VI).

6.4 CHROMIUM SENSITIVITY

Sensitivity to chromium compounds has been widely reported in the research literature dating back to 1869 when Delpech and Hillairet (cited in Joules, 1932) described five cases of asthma in chromium workers. Cirla (1985) reviewed the literature on chromium sensitivity and noted that asthma, which can be a manifestation of hypersensitivity, could be induced by exposure to chromium compounds during chrome plating, galvanic processes, and stainless steel welding. Although (Cr(VI) is considered the sensitizing determinant of chromium skin allergy, Cr(III) has also been associated with sensitization because of its ability to bind and denature proteins. However, in the work reviewed in Cirla (1985), asthmatic symptoms/ sensitization were seen only in some subjects exposed to Cr(VI). Data for two subjects are presented as examples by Cirla (1985). The Cr(III) exposure (compound not stated) of 260 $\mu g/m^3$ for 30 min had virtually no effect, whereas exposure to 150 $\mu g/m^3$ Cr(VI) (unknown compound) for 30 min "provided immediate asthmatic reactions".

Using radioactive compounds, Fitzgerald (1982) noted that Cr(III) binds protein haptens and cannot penetrate the skin membranes, whereas Cr(VI) can penetrate skin membranes and is reduced to Cr(III). When Cr(III) is injected subcutaneously, it may cause a reaction such as dermal irritation (Naruse et al., 1982). Burrows (1984) concluded from a review of the literature that in those people with eczematous skin disease, 5- to 10 percent were sensitive to dichromate (CrVI) compounds as determined by a positive patch test; the ratio of male to female respondents was 3 to 1.

The prevalence of chromium sensitivity was studied more extensively by Peltonen and Fraki (1983). Two groups were studied: one consisted of 822 healthy volunteers, including 110 workers who had some exposure to chromium; the second group consisted of 2,981 hospital patients, including 499 with possible occupational exposure to chromium. In the first group, chromium allergy, as determined by a positive two-day patch test with 0.5 percent potassium dichromate (CrVI), was observed in 10 of the 110 chromium-exposed workers (9 percent). Among 712 persons without known occupational exposure, four showed positive reactions (0.6 percent). In this 5-year patient study (Peltonen and Fraki, 1983) 6.8 percent of the men and 2.8 percent of the women reacted positively. Among the occupationally exposed segment, 20 percent of the men and 8 percent of the women responded; only 1.3 percent of a separate group composed of 390 patients with atopic dermatitis responded. Chromium sensitivity is, thus, mainly an occupational problem. In contrast to nickel, chromium metal does not sensitize people, which makes it unlikely for members of the general population to be sensitized. One source may be shoes if Cr(VI) has been used for tanning.

6.5 DEVELOPMENTAL TOXICITY

It was concluded earlier that injections to experimental animals of Cr(III) or Cr(VI) compounds could cause embryotoxic and teratogenic effects (U.S. Environmental Protection Agency, 1984a). At that time there were no data on effects of orally administered chromium. Trivedi et al. (1989) gave pregnant mice potassium dichromate in drinking water daily during the gestation period. The concentrations in the water were 250, 500 and 1000 mg/l as potassium dichromate, the ingested daily amounts were calculated to be 1.8, 3.6 and 7.0 mg

of Cr(VI) respectively, which corresponds to about 60, 120 and 200 mg/kg b.w. Dose-dependent effects on fetal development such as decreased litter sizes, malformations and increased resorption were noted. The doses were very high and there is still a lack of data on teratogenic effects at low levels of oral exposure.

6.6 OTHER EFFECTS

With the exception of the kidney there are no conclusive data that indicate that in human beings internal organs are affected by absorbed chromium (VI) compounds (U.S. Environmental Protection Agency, 1984a; World Health Organization, 1988).

6.7 FACTORS MODIFYING TOXICITY

Ginter et al. (1989) studied the effect of ascorbic acid status on the toxicity of Cr(VI). They used guinea pigs, which like human beings cannot synthesize this vitamin. In animals with a low intake of ascorbic acid for 8 weeks, regarded as marginally deficient, injections of potassium dichromate (8 mg/kg b.w.) caused significantly more chromosome aberrations in bone marrow cells than in animals with a high intake of ascorbic acid. The tissue levels of ascorbic acid were about 10 times higher. In another experiment, guinea pigs were given potassium dichromate in drinking water (28 mg/l) for 24 days. The daily intake of the dichromate was estimated to be about 8 mg/kg b.w. Dichromate exposure did not cause any changes in tissue concentrations of ascorbic acid in animals on low or high intake of ascorbic acid, but there were significant decreases in some liver microsomal enzyme activities in the ascorbic acid-deficient animals. In this study, the animals on high intake of ascorbic acid had tissue concentrations of ascorbic acid 2-3 times higher than those seen in the deficient animals. In the bone marrow there were no effects in animals on high intake of ascorbic acid, whereas in deficient animals there was a significant increase in micronuclei in polychromatic erythrocytes, indicating a mutagenic effect.

6.8 SUMMARY AND CONCLUSIONS

Recent data (Lindberg and Hedenstierna, 1983) support earlier findings (Cohen et al., 1974; Lucas and Kramkowski, 1975) that in occupational settings air concentrations above 2 $\mu g/m^3$ of Cr(VI) as chromic acid are highly irritating to the nasal mucosa and can induce morphological changes. Even average concentrations of 1-2 μg Cr(VI)/m^3 seem to be irritating, but peak exposures are probably of great importance. Chromic acid is a highly soluble and reactive compound, which may represent the worst case. Chromates and dichromates will show irritating properties related to water solubility. Slight changes in lung function were seen during work at exposure levels of 2-20 μg Cr(VI)/m^3. These changes are probably reversible since it was not possible to demonstrate long-term effects (Lindberg and Hedenstierna, 1983).

Reversible effects on the kidney seem to appear at exposure levels above 4 μg Cr(VI)/m^3 (Lindberg and Vesterberg, 1983b), corresponding to 10-15 μg Cr/l in urine.

From the available data, a LOEL of 1 μg Cr(VI)/m^3 is the best estimate for occupational exposure to chromic acid. At that level there should be no systemic effects, only minor irritation of the upper airways.

With regard to the general population a life-time exposure to a level of 0.1 μg Cr(VI)/m^3 should not cause irritation of the airways or other local or systemic toxic reactions. That estimate is based on the properties of chromic acid, which in ambient air will be only a minor part of the total chromium, Cr(III) being the most common form.

However, the reduction of Cr(VI) to Cr(III), which has a long retention time in the lungs, may lead to an accumulation of Cr(III) in the lungs, and it is reasonable to lower the acceptable level to 0.01 μg Cr(VI)/m^3. Since it is very difficult to determine small amounts of chromium species in air, a level of 0.05 μg of total chromium per m^3 is recommended. This will ensure low concentrations of Cr(VI) since Cr(III) is the major form of total chromium in air.

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